# CHEMISTRY OF NATURAL PRODUCTS

**SUMMARY**

THESIS SUBMITTED FOR THE DEGREE OF

**Doctor of Philosophy**

IN

CHEMISTRY

**MUSHTAQ AHMED KHAN**

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

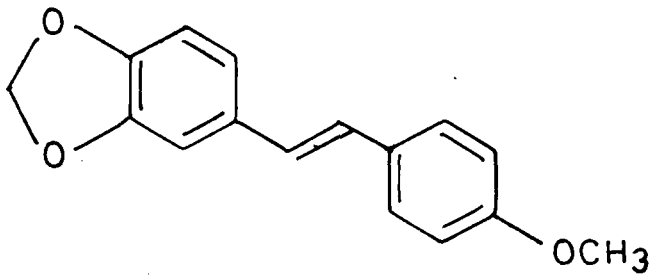
1985-86

## SUMMARY

The constituents of a number of plants were studied under a scheme for investigation of indigenous medicinal plants. Interesting constituents were isolated from four of these and structural studies on these form the basis of the work presented in the thesis.

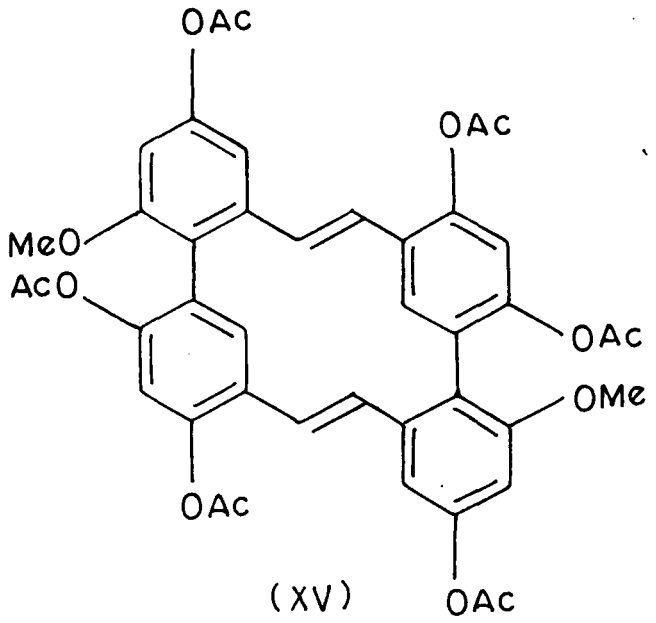
Gnetum ula (Gnetaceae)

The acetone extract of the plant was subjected to column chromatography over silica gel. Some of the products isolated from this chromatography have been discussed in another thesis*. During the course of the present work two new compounds have been isolated and assigned the structures (XI) and (XV). Besides this structure (IV) assigned earlier to the trihydroxy-monomethoxy stilbene has now been revised to (VI).

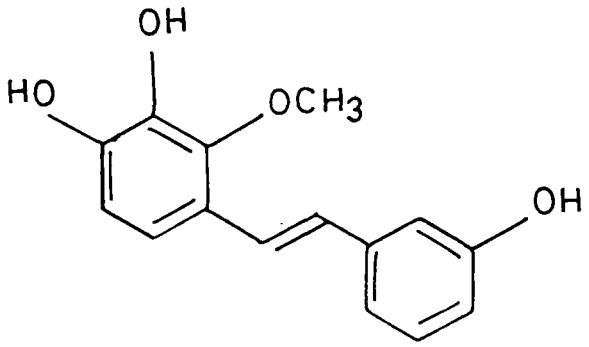



( XI )

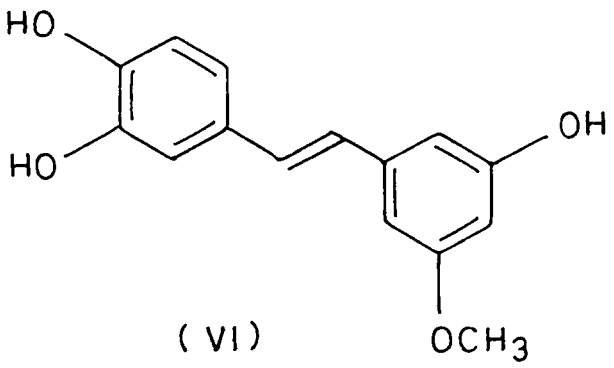
*Ph.D. thesis of Dr. Jamal Ahmad (1981), Department of Chemistry, Aligarh Muslim University, Aligarh, India.




(XV)



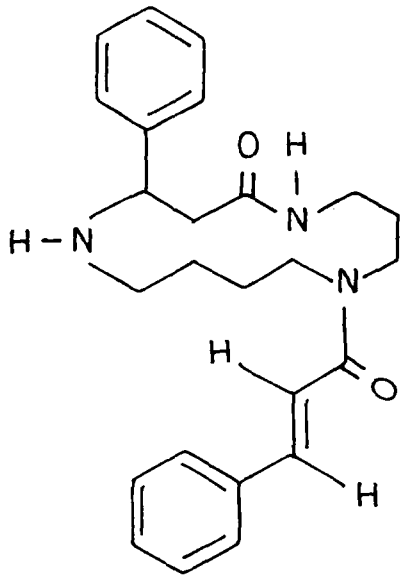

(IV)




(VI)

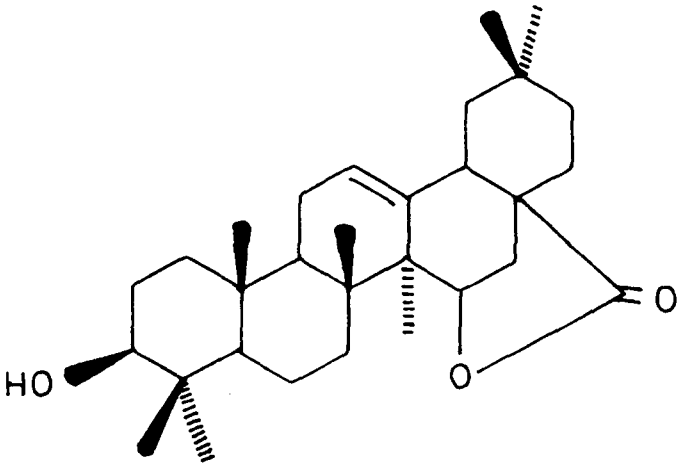
Maytenus emarginata (Celastraceae)

Maytenus species came into prominence with the isolation of compounds having antileukemic and antitumor activity. Extraction of the leaves of Maytenus emarginata collected from South India afforded crude alkaloid in poor yields which was shown by TLC to be a mixture of several components. Further chromatographic separation yielded only one compound in a sufficient degree of purity for identification. Spectral data shows it to be celacinnine (XVII) with traces of celallocinnine.




( XVII )

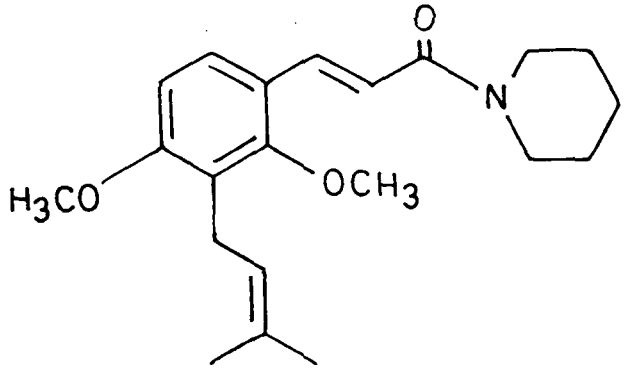
The roots of the plant afforded the β-amyrin lactone (XXII) which was isolated by Chinese workers from Tripterygium wilfordii in 1984 but a direct comparison could not be made.




( XXIII )

Excoecharia agallocha (Euphorbiaceae)

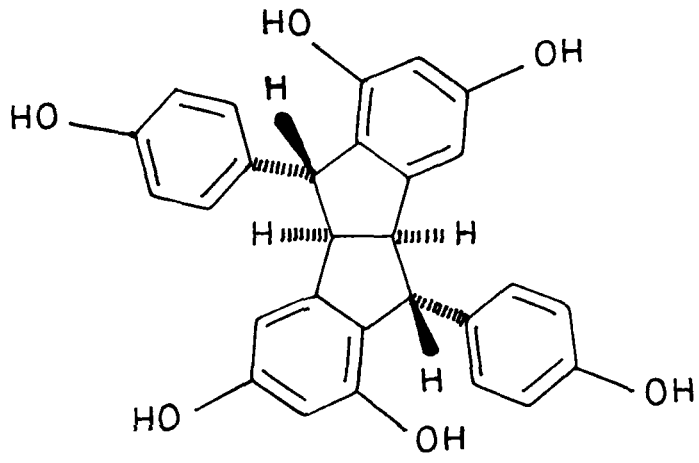
A piperidine alkaloid was isolated from it earlier but spectral data did not completely define its substitution pattern. Comparison with the compound now synthesised shows it to have the structure (XXV).




( XXV )

Cissus pallida (Vitaceae)

This plant was collected from the same region from which Maytenus emarginata was obtained. The plant extract was found to contain a high molecular weight phenol which has been identified as the new stilbene oligomer (XXXIV).




( XXXIV )

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1985-86

To My Parents

PHONE-Office : 5515

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH, U.P., INDIA

*No.* _____ *Date* _____

The work described in this thesis was carried out by the candidate, Mushtaq Ahmed Khan, personally. It has not been submitted for any other degree, either of this or anyother University.

Asif Zaman
(Prof. Asif Zaman)

## ACKNOWLEDGEMENT

It is with immense pleasure that I place on record my indebtedness to Prof. Asif Zaman under whose inspiring guidance this work was carried out.

I would like to thank Prof. M.S. Ahmad, Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh for his helpful discussions in solving research problems and for providing research facilities.

With equally sincere feelings, I extend my thanks to Dr. K.M. Shamsuddin and Dr. S. Prakash for their valuable suggestions through out the course of this work.

I am grateful to Prof. Atta-ur-Rahman, H.E.J. Research Institute of Chemistry, Karachi for providing some NMR and mass spectra.

Thanks are also due to my colleagues for their co-operation and cheerful companionship.

Financial assistance from the UGC, CSIR and the CCRUM is gratefully acknowledged.

MKhan

(Mushtaq Ahmed Khan)

CONTENTS

# INTRODUCTION

# Introduction

The thesis describes the isolation and identification of the constituents of four plants viz. Gnetum ula, Maytenus emarginata, Excoecharia agallocha and Cissus pallida. The work on Gnetum ula was in continuation of earlier studies and led to the characterisation of a new stilbene and revision of the structure of a trihydroxymonomethoxy stilbene. The structures of both these stilbenes were further confirmed through synthesis. The ethyl acetate eluate from the chromatography of the plant extract afforded a product which resisted crystallisation and had to be purified through acetylation. The molecular weight of the acetate showed it to be a stilbene dimer for which a tentative structure is suggested. It may be pointed out here that stilbene oligomers have also been isolated from other plants of the family Gnetaceae.

Maytenus emarginata was taken up because of the isolation of antitumor compounds, maytansinoids, from other species. Though it contains alkaloids the yield of the crude mixture of several alkaloids was only about 0.08% of the dry weight of leaves. Separation through chromatographic methods proved extremely tedious and ultimately not very successful. Separation of maytansinoids has usually been affected through

HPLC but this facility was not locally available. Only two alkaloids in comparatively pure form could be obtained of which one has been identified as celacinnine with trace amounts of celallocinnine.

A triterpene lactone, β-amyrin and β-amyrone were isolated from the roots of Maytenus emarginata. The lactone was identified from its spectral data as olean-12-en-28 oic acid, 3,15-dihydroxy-γ-lactone isolated in 1984 from Tripterygium wilfordii by Chinese workers.

Excoecharia agallocha too was investigated in this laboratory earlier and a cinnamoyl piperidide had been isolated from it. Since the substitution pattern of the compound was not unambiguously settled by its NMR spectrum the biogenetically more likely structure was synthesised and comparison showed it to be identical with the natural sample.

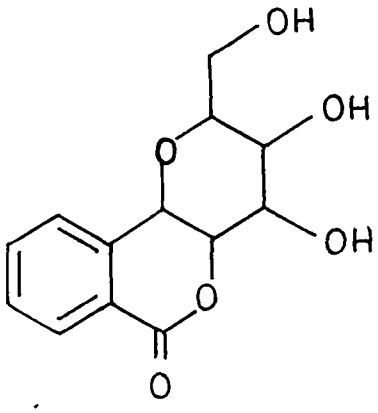
Cissus pallida, like Maytenus emarginata, was collected from the hilly regions adjoining Hyderabad city in South India. The alcohol extract afforded dimethyl terephthalate, β-sitosterol, β-sitosterol glucoside, arjunolic acid and a fifth compound having the molecular formula $C_{28}H_{22}O_6$ which has been shown to be a new resveratrol dimer incorporating the unusual dibenzopentalene ring system.

# D I S C U S S I O N

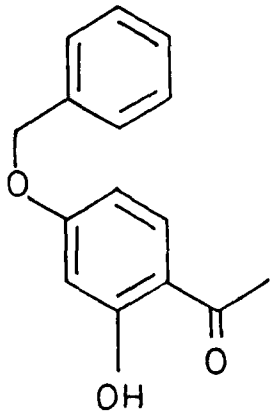
Gnetum ula (Gnetaceae)

The family Gnetaceae has been of interest to botanists because of the morphology of its vessels which deviates from that of other gymnosperms[1]. It comprised initially of two genera, Gnetum and Ephedra[2] but Ephedraceae has now been given the status of a separate family[1]. Members of both families contain alkaloids and ephedrine has also been isolated from Gnetum indicum[3].

Gnetum ula was collected from Goa (West India) where it grows in abundance though in areas not easily accessible. TLC examination of the crude extract revealed the presence of a number of fluorescent substances but none of these responded to specific alkaloidal reagents. The major components of the extract were isolated and identified as the isocoumarin bergenin (I), the acetophenone derivatives (II)

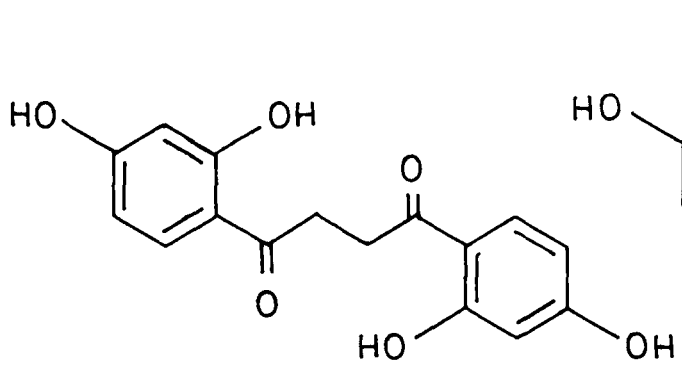



(I)

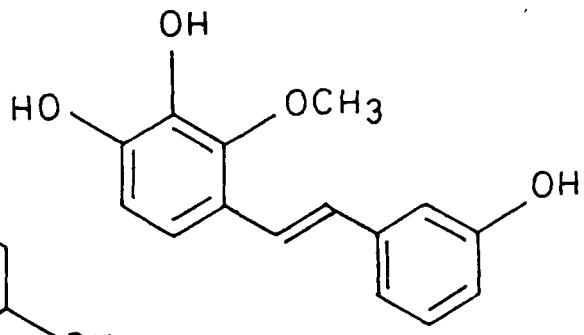



(II)

and (III) and a stilbene to which structure (IV) was initially assigned[4].



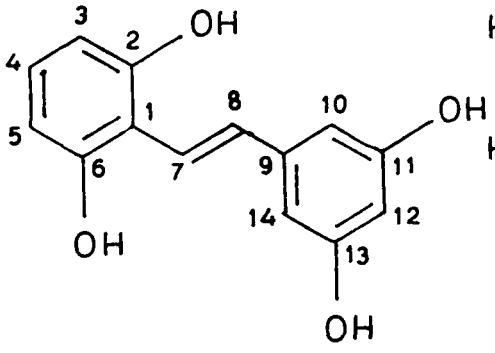

(III)



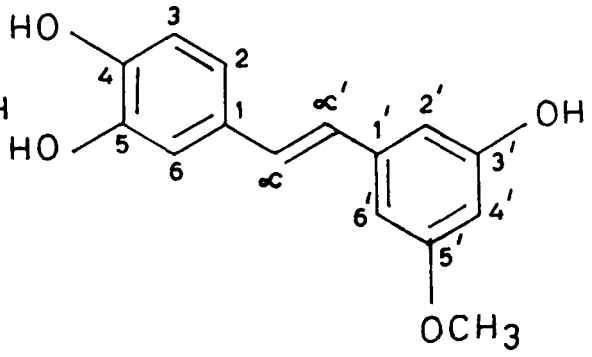

(IV)

Further work on the constituents of Gnetum ula led to the isolation of a new stilbene, gnetol, which was characterised unambiguously as (V) on the basis of solvent induced shifts and decoupling experiments at 360 MHz followed by synthesis[5]. The findings of the NMR studies on gnetol cast doubts on the assignments made earlier in the 90 MHz NMR spectrum of the methoxy stilbene (IV) and forced a revision of its structure to (VI)[6]. The evidence which favoured the structure (IV) originally assigned is briefly summarised and the revised structure is discussed below.

The mass spectrum of the dihydroderivative of the methoxy stilbene, $M^{+\cdot}$ at m/z 260, shows peaks at 232 (60%), 138 (95%), 123 (50%) and 107 (100%). If the two rings are evenly oxygenated fission of the molecular ion should give rise to benzylic cations of about equal intensity at m/z 137 and 123. The substantial difference in abundance of the two




(V)



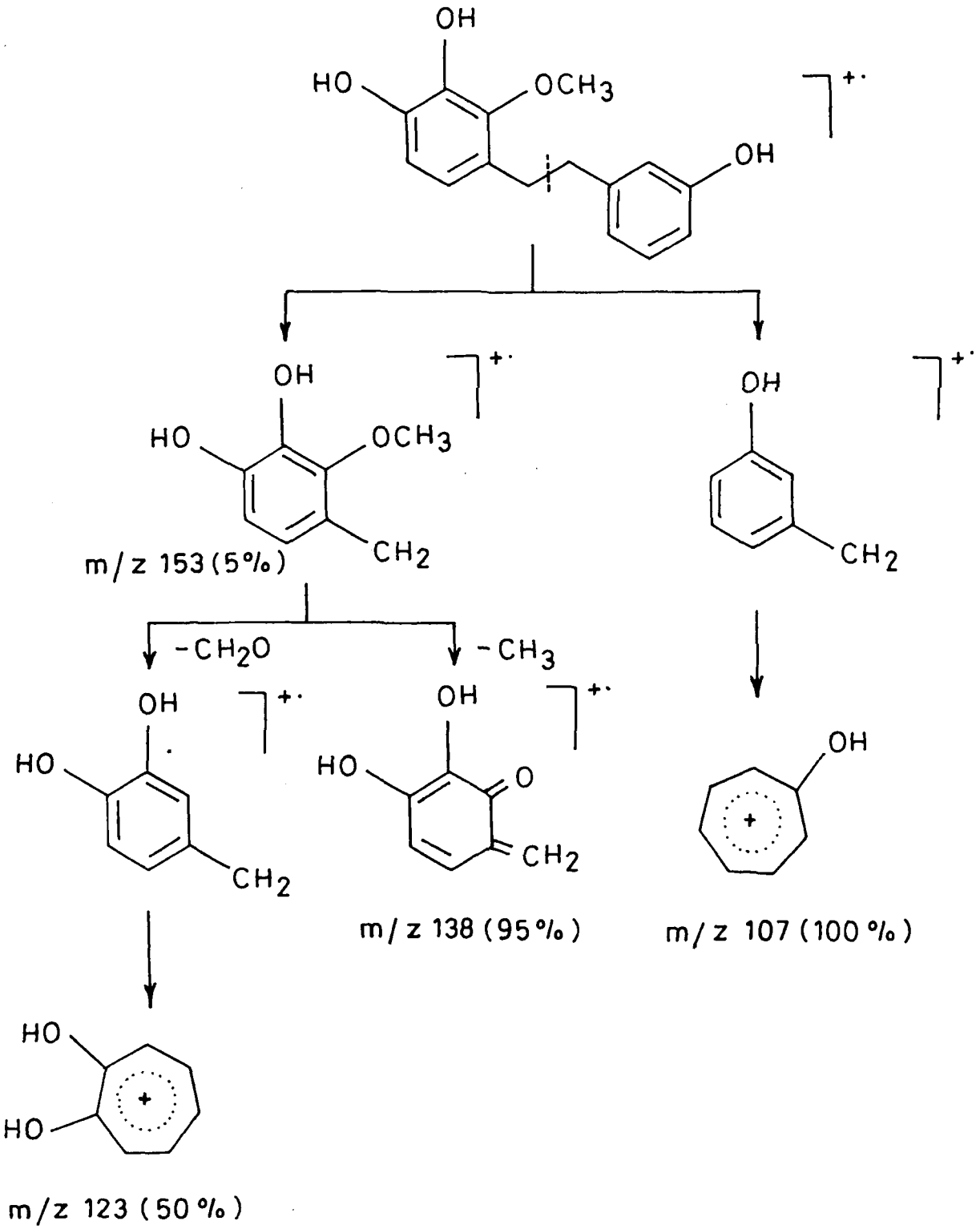

(VI)

fragments coupled with the presence of an abundant ion at m/z 107 seemed to rule out this possibility. As against this if one ring carries a single hydroxyl group, the other the methoxyl and the remaining hydroxyls, the peaks at m/z 137, 123 and 107 and their relative intensities are readily

explained as shown in scheme I on the basis of the structure assigned earlier.

The more compelling reason for assignment of structure (IV) to the methoxy stilbene was, however, the presence in its 90 MHz NMR spectrum of the ortho coupled doublets at 6.78 and 7.40 which is possible only if one ring is tetrasubstituted (Fig. 1). Since the spectrum was measured in DMSO-$d_6$ owing to its complete insolubility in $CDCl_3$ the wide difference in the position of these signals was not considered critical. The relative position assigned to the methoxyl- and to the hydroxyl in the monooxygenated ring- followed from the failure of the methoxy stilbene to undergo cyclisation to a benzofuran and lack of any indication of AA'BB' pattern in the NMR spectrum.

The 360 MHz NMR spectrum of gnetol (Fig. 2) shows a triplet at 6.08 which corresponds in appearance to the signal at 6.16 in the 90 MHz NMR spectrum of the methoxy stilbene. The results of benzene induced shifts in the NMR spectrum of gnetol leave no doubt that this signal arises from resonances of the C-12 ≡ C-4' hydrogen. Accordingly part structure (VII) must be present in the methoxy stilbene also which makes it impossible to account for the ortho coupled


OH
HO
OCH3
OH
+·
OH
HO
OCH3
CH2
+·
OH
CH2
+·
m/z 153 (5%)
-CH2O
-CH3
OH
HO
CH2
+·
OH
HO
O
CH2
+·
OH
+
m/z 138 (95%)
m/z 107 (100%)
HO
HO
+
m/z 123 (50%)

scheme I

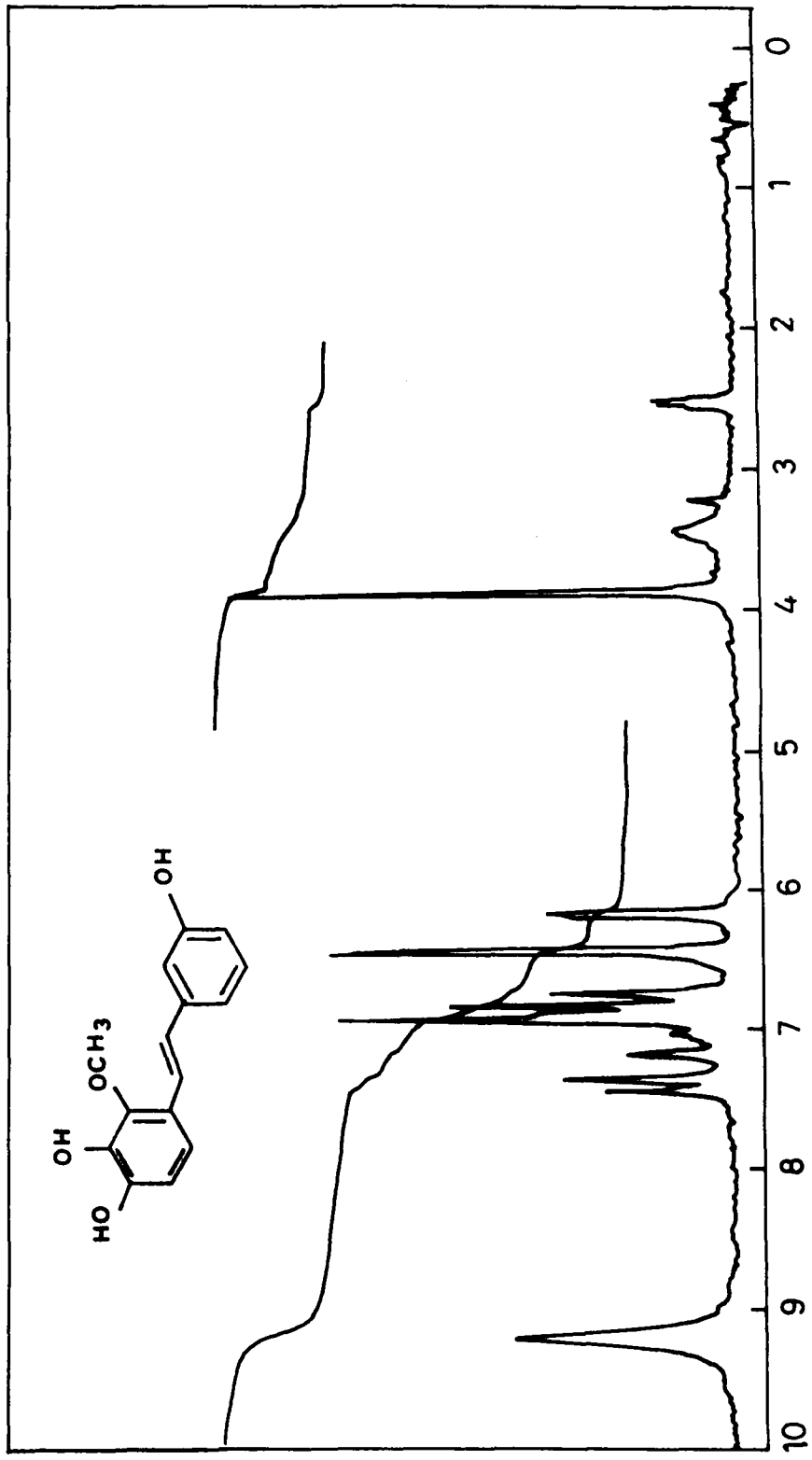
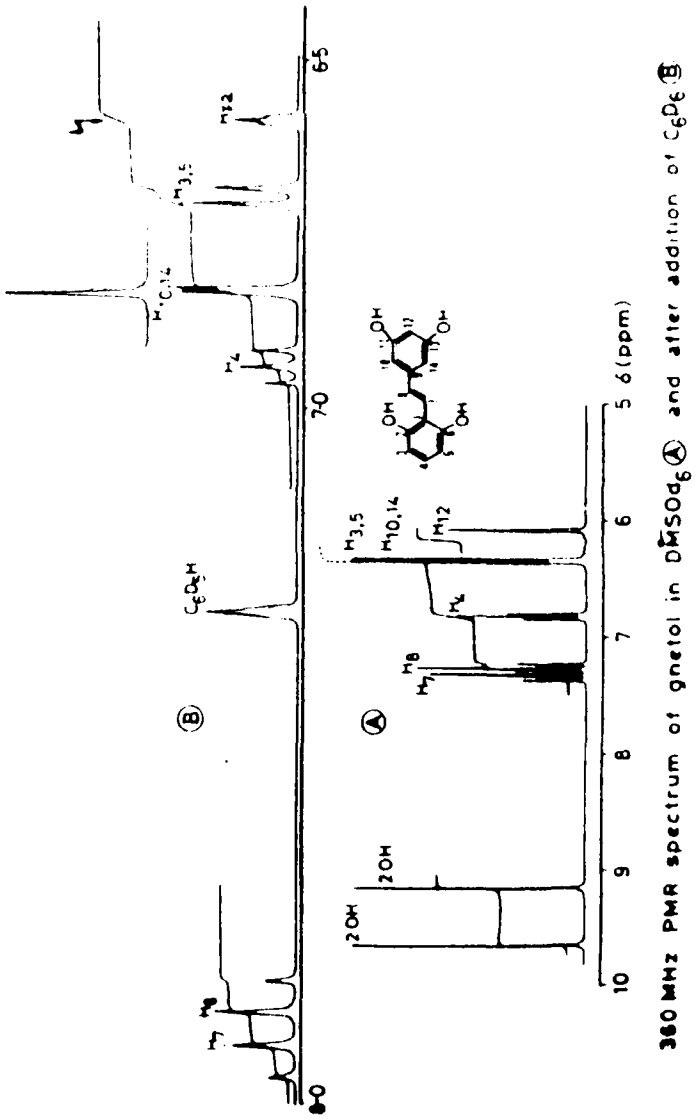
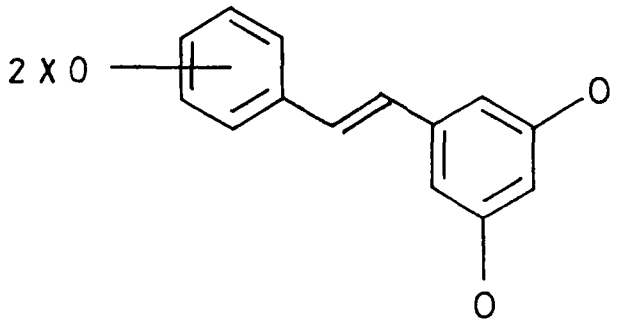



FIG. 1




360 MHz PMR spectrum of gnetol in $DMSOd_6$ (A) and after addition of $C_6D_6$ (B)

FIG.2




( VII )

doublets. This made it necessary to re-examine the problem and for this purpose the available amount of the methoxy stilbene was repeatedly crystallised and a fresh 100 MHz NMR spectrum (Fig. 3) of it was obtained. This showed, surprisingly, no low field doublet and it must, therefore, be assumed that this doublet belonged to an impurity which was eliminated on further crystallisation. The explanation is justified by the presence of a residual signal at the same value in the spectra obtained subsequently but it is difficult, nevertheless, to account for the absence of signals of the remaining protons of this impurity in the earlier spectrum. The 270 MHz NMR spectrum (Fig. 4) kindly furnished by the Indian Institute of Science, Bangalore shows distinctly only one ortho coupled doublet at 6.75 and a triplet like signal at 6.12, signals of the other protons are not clearly

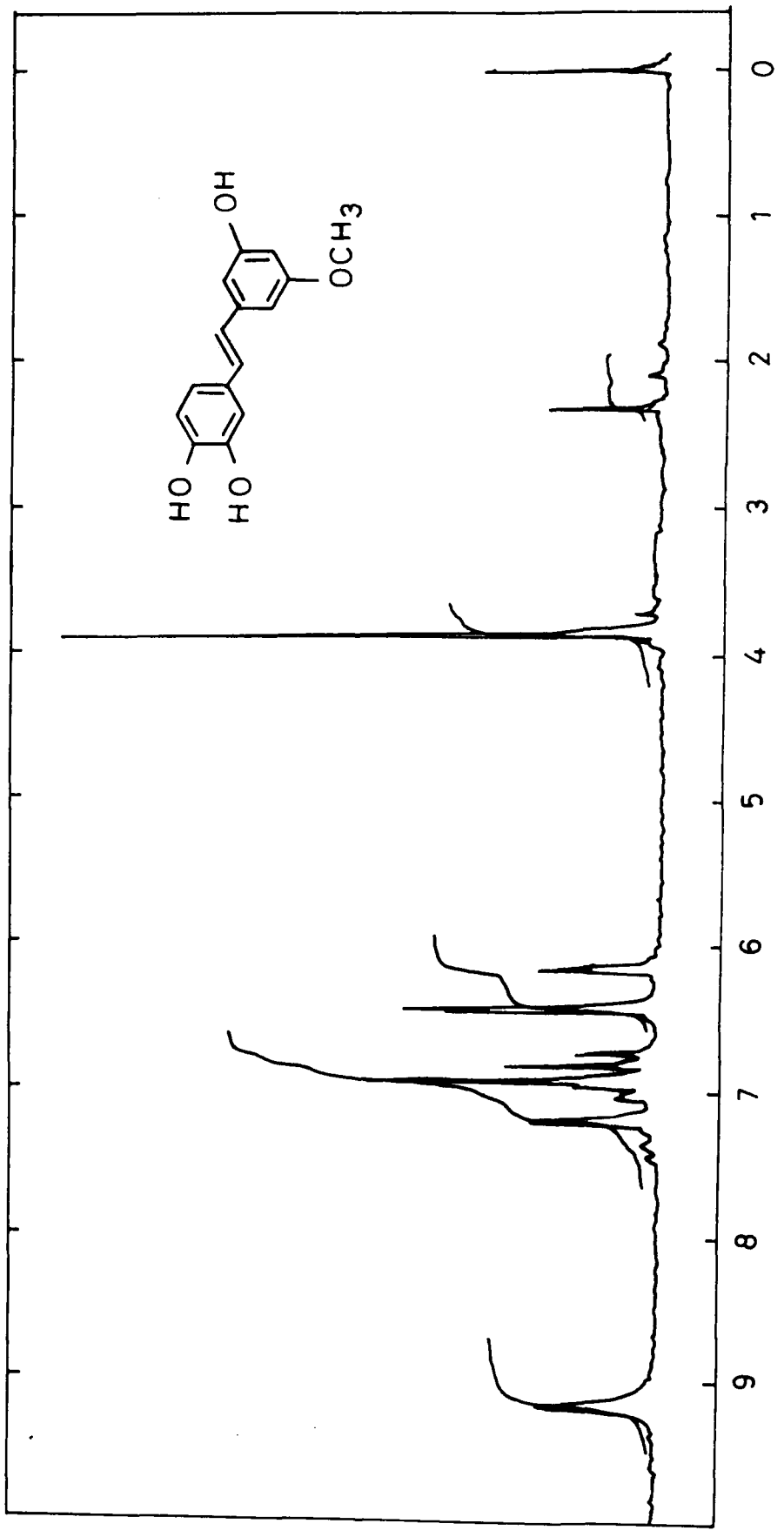



FIG. 3

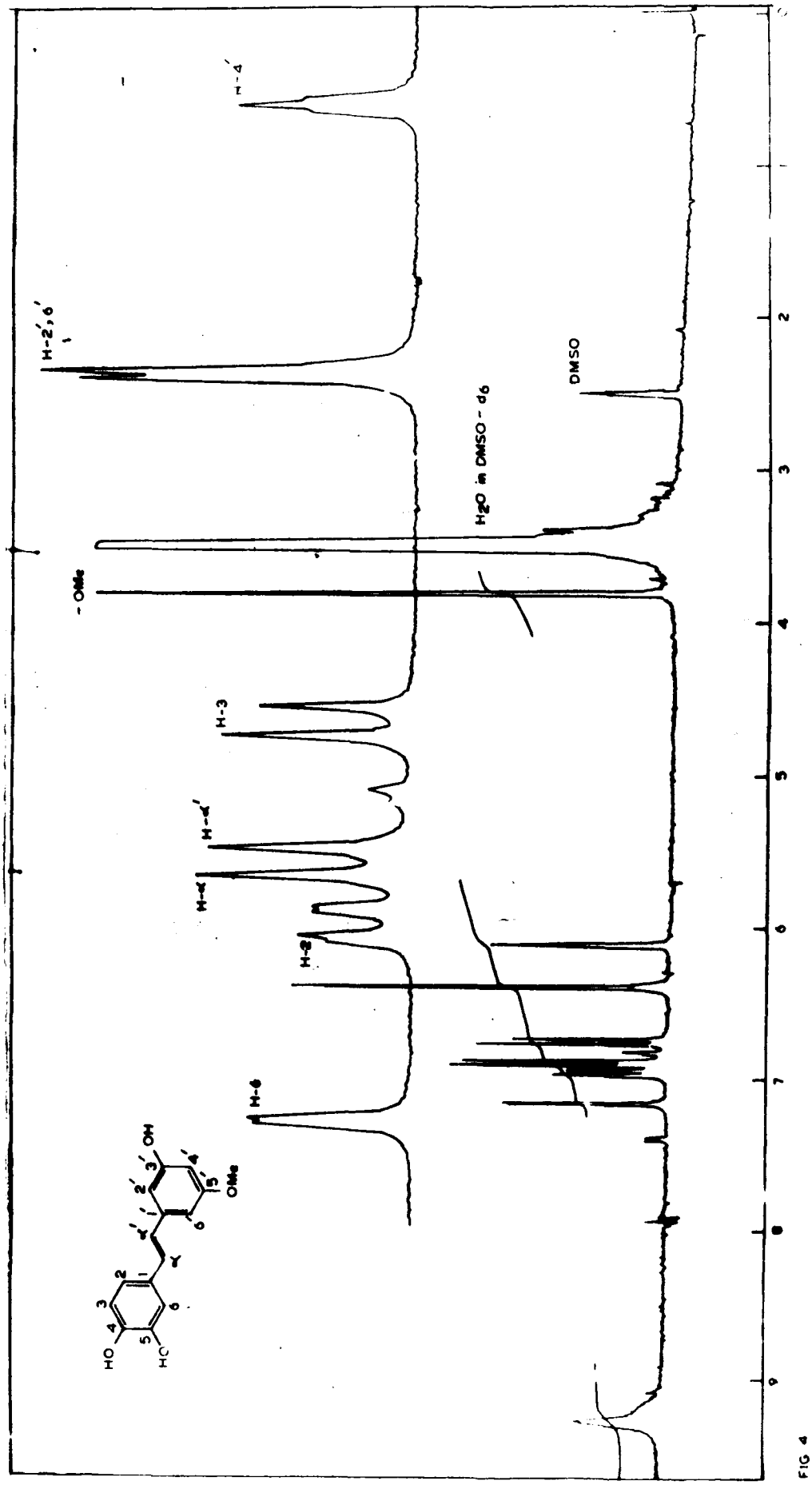
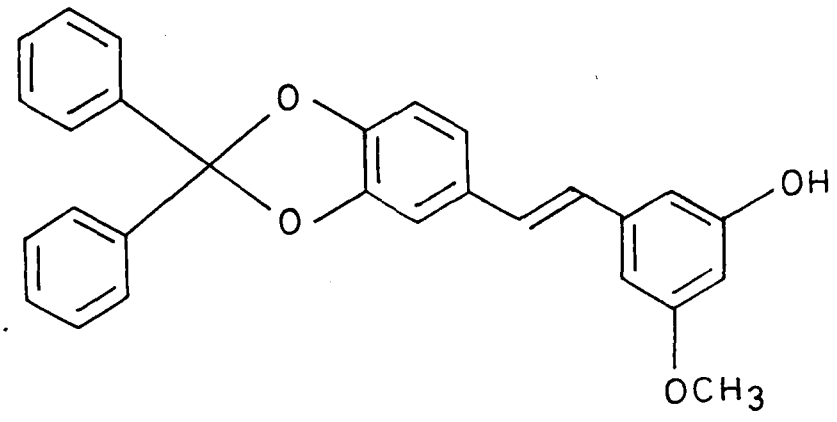

H-2',6'
H-4'
-OMe
H-3
H-4'
H-4
H-2
H-6
H2O in DMSO - d6
DMSO
1
2
3
4
5
6
7
8
9
HO
OH
OMe
OMe

FIG 4

resolved but measurement after addition of benzene-$d_6$ afforded much better resolution. The NMR spectrum (Fig. 5) after addition of benzene-$d_6$ shows the signal of the C-4' hydrogen as a clear triplet, the (2H) doublet at 6.53 accounts for the two remaining protons of this ring which are equivalent. The olefinic protons give rise to doublets at 6.96 (J=17 Hz) and 7.03 (J=17 Hz) and the C-6 hydrogen to a meta coupled doublet at 7.16. The multiplet of C-2 hydrogen is, however, not completely resolved owing to overlap with signals of the olefinic protons. The dark green ferric colour and formation of a cyclic derivative (VIII) with diphenyldichloromethane[7] places the methoxyl definitely in the resorcinol ring.

In the light of the evidence discussed above the mass spectral fragmentation initially assumed for the dihydroderivative of the methoxy stilbene must be revised as shown in scheme II.




( VIII )

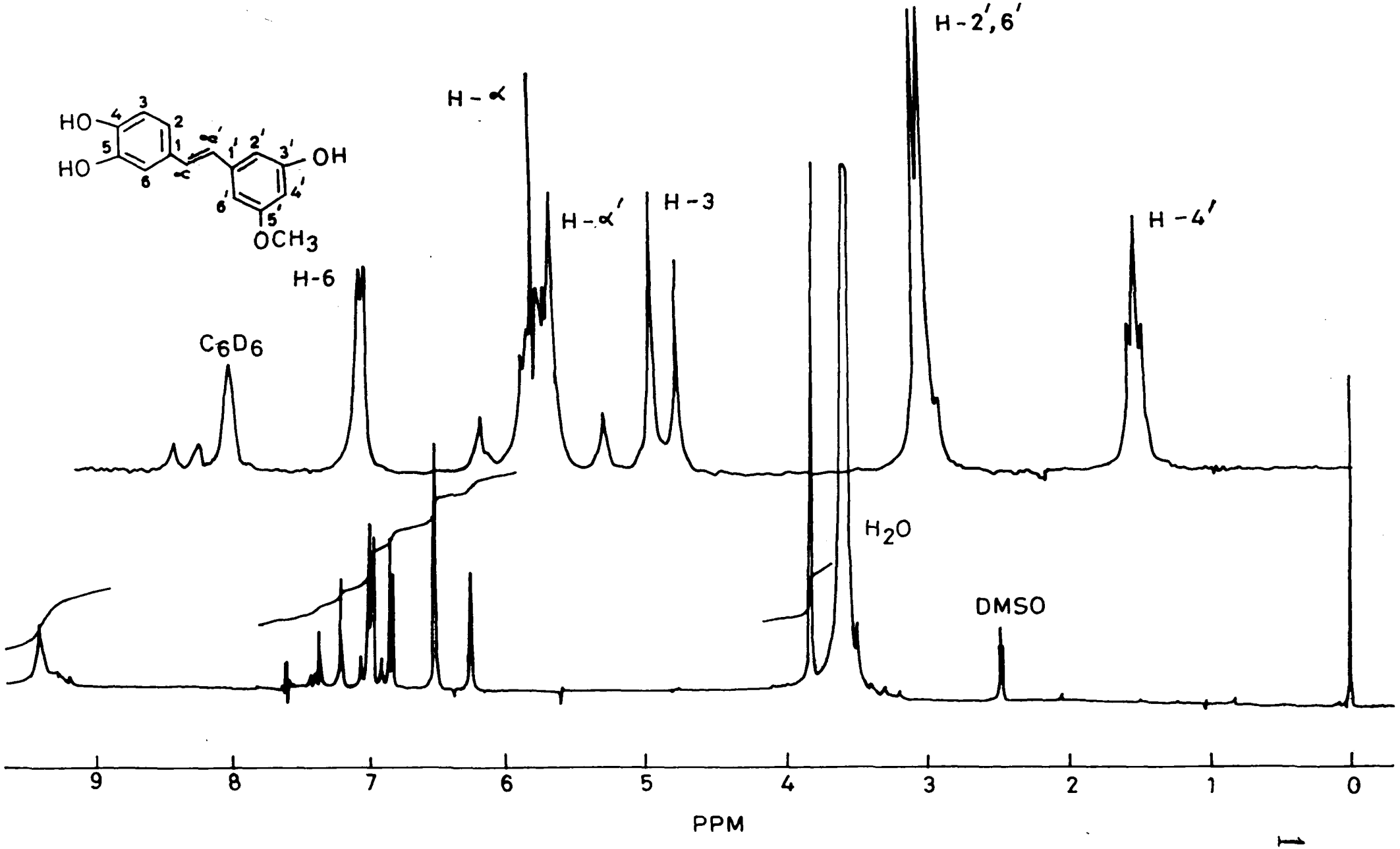
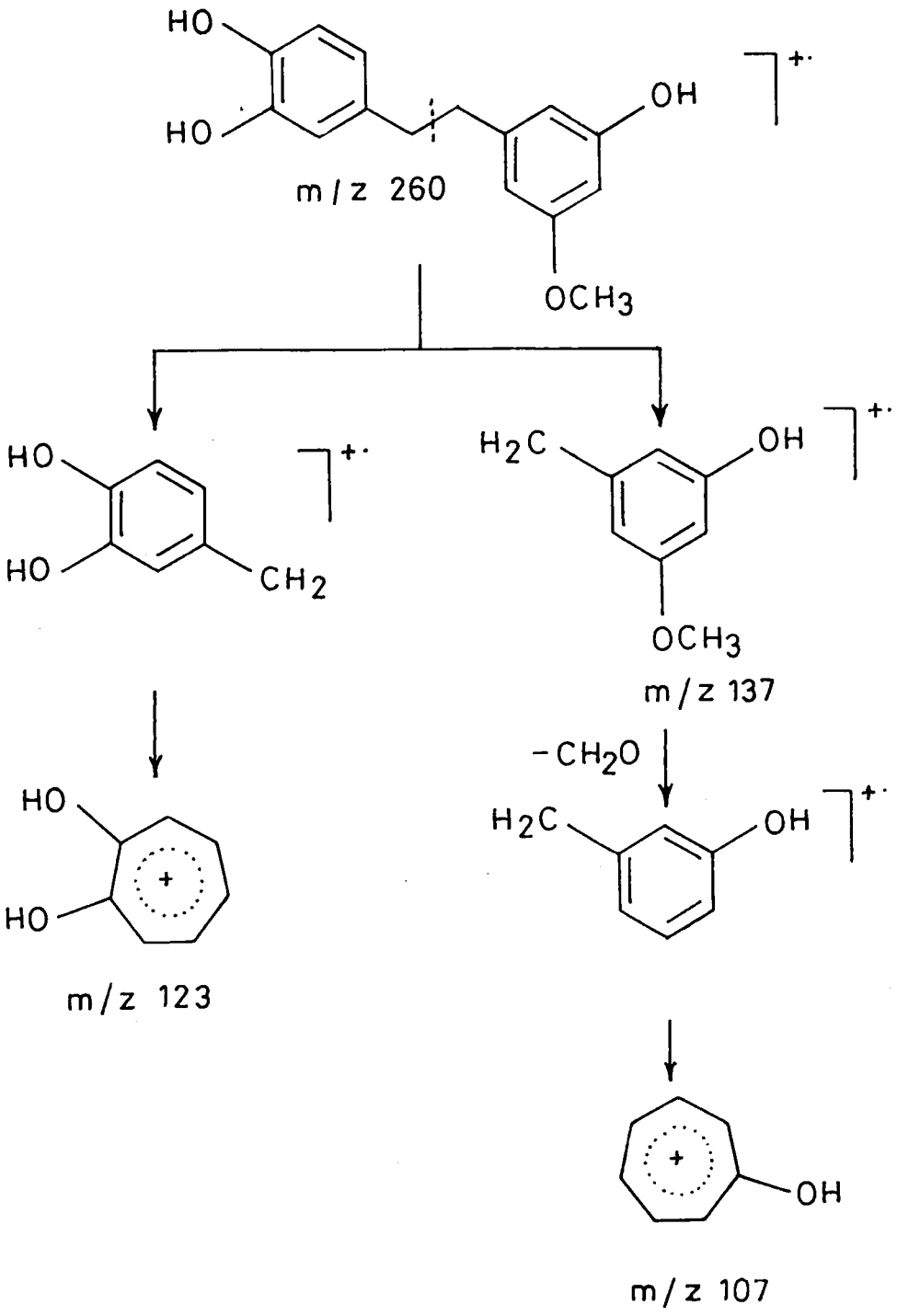


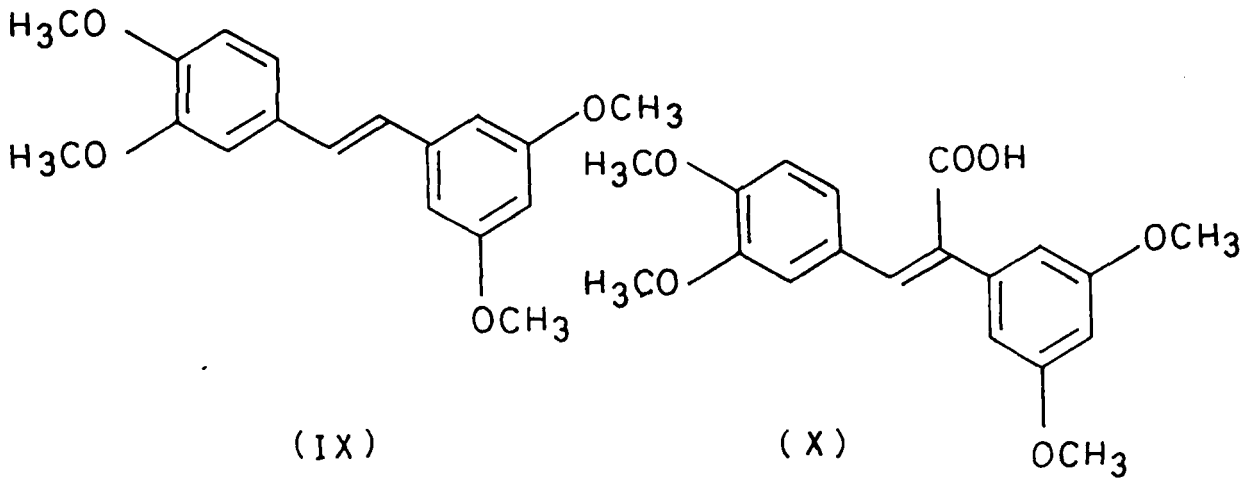

FIG. 3


HO
HO
OH
OCH3
m/z 260
HO
HO
CH2
H2C
OH
OCH3
m/z 137
-CH2O
HO
HO
m/z 123
H2C
OH
OH
m/z 107

scheme II

Synthesis of 3,3',4,5'-tetramethoxy-trans-stilbene

Corroborative evidence for structure (VI) was obtained through a routine stilbene synthesis. 3,5-dimethoxyphenyl acetic acid could not be obtained commercially and was prepared through Arndt-Eistert synthesis[8]. It was condensed with 3,4-dimethoxybenzaldehyde in presence of piperidine[9]. The product was separated into neutral and acidic fractions. The neutral fraction was found to be a mixture which was purified through column chromatography to give 3,3',4,5'-tetramethoxy-trans-stilbene (IX). The acidic fraction contained the corresponding α-carboxylic acid (X) which was decarboxylated to (IX) by heating with $CuCO_3$ in quinoline[10]. 3,3',4,5'-tetramethoxy-trans-stilbene was found identical (Co-TLC, IR, NMR) with the permethylation product of the natural sample.




(IX) (X)

Subsequent collection of more plant material enabled the isolation of one more compound designated as gnetin (XI)[6] which was characterised as follows.

Gnetin

This compound was obtained through extensive chromatography of the intricate mixture eluted by benzene. It crystallised from acetone-petroleum ether as light yellow plates, m.p.121-122°, and TLC examination to check its purity also established that it was ferric negative. Fluorescence under UV light and spectral data such as absence of carbonyl band in the IR (Fig. 6) and high wave length absorption in the UV (Fig. 7)[11] suggested that like other compounds isolated from Gnetum ula, gnetin was also a stilbene. The 60 MHz NMR spectrum (Fig. 8) of gnetin (XI) shows nine aromatic or deshielded olefinic protons and evidence of the presence of methoxyl and methylenedioxy groups. The mass spectrum indicates a molecular weight of 254 corresponding to $C_{16}H_{14}O_3$. Exposure to hydrogen/Pd/C gave the dihydrostilbene (XIII) as an oil, the 60 MHz NMR spectrum (Fig. 9) shows the two benzylic methylenes as (4H) singlet at 2.80. Of the signals of aromatic protons in gnetin the AA'BB' doublets centred at 6.83 and 7.40 are clearly visible. The olefinic protons

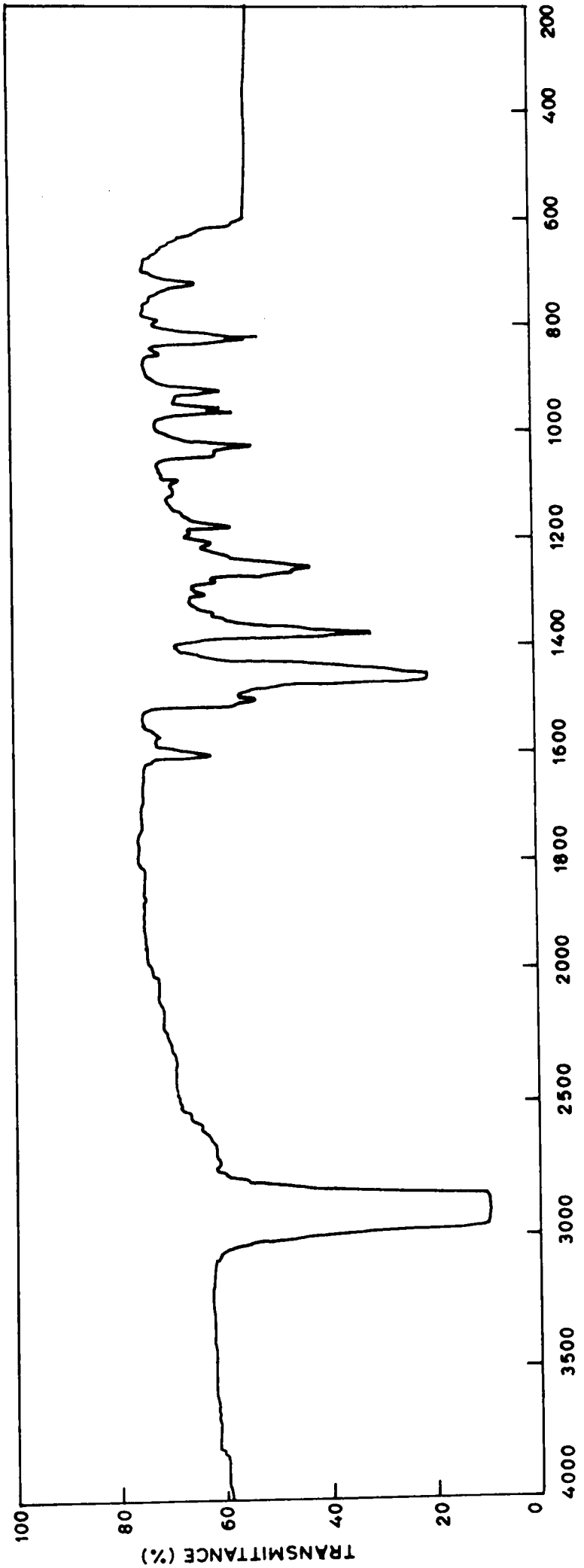

TRANSMITTANCE (%)
100
80
60
40
20
0
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
200

FIG. 6

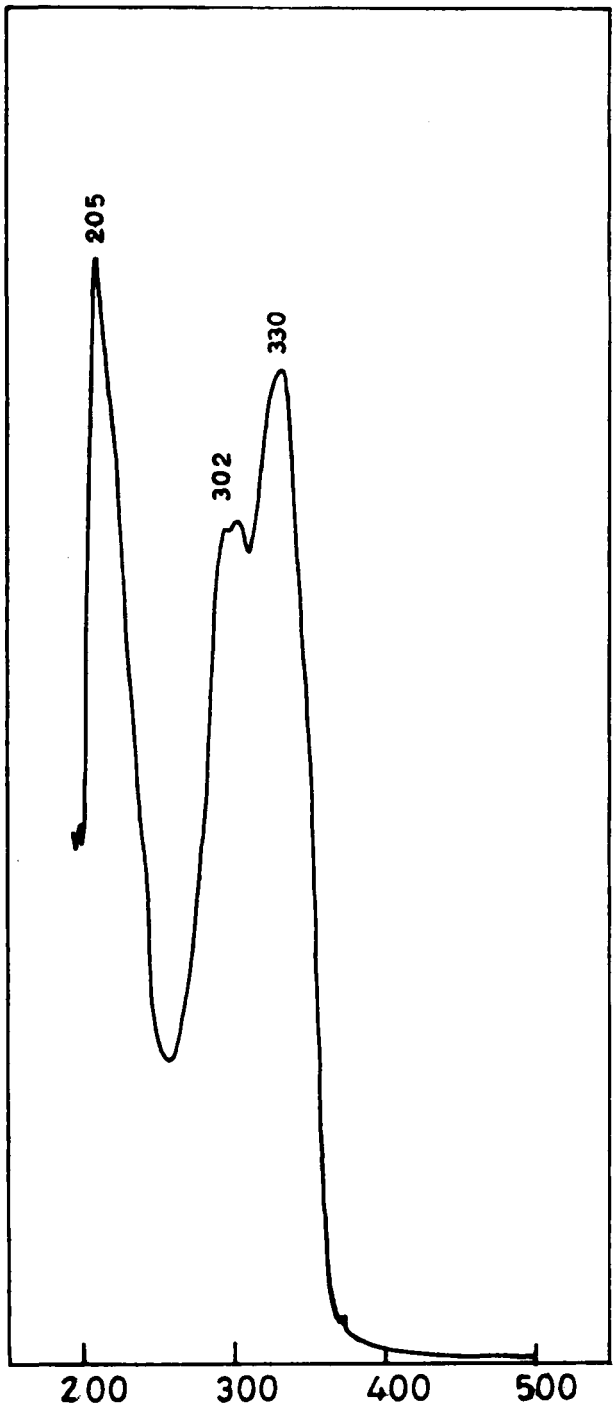



FIG. 7

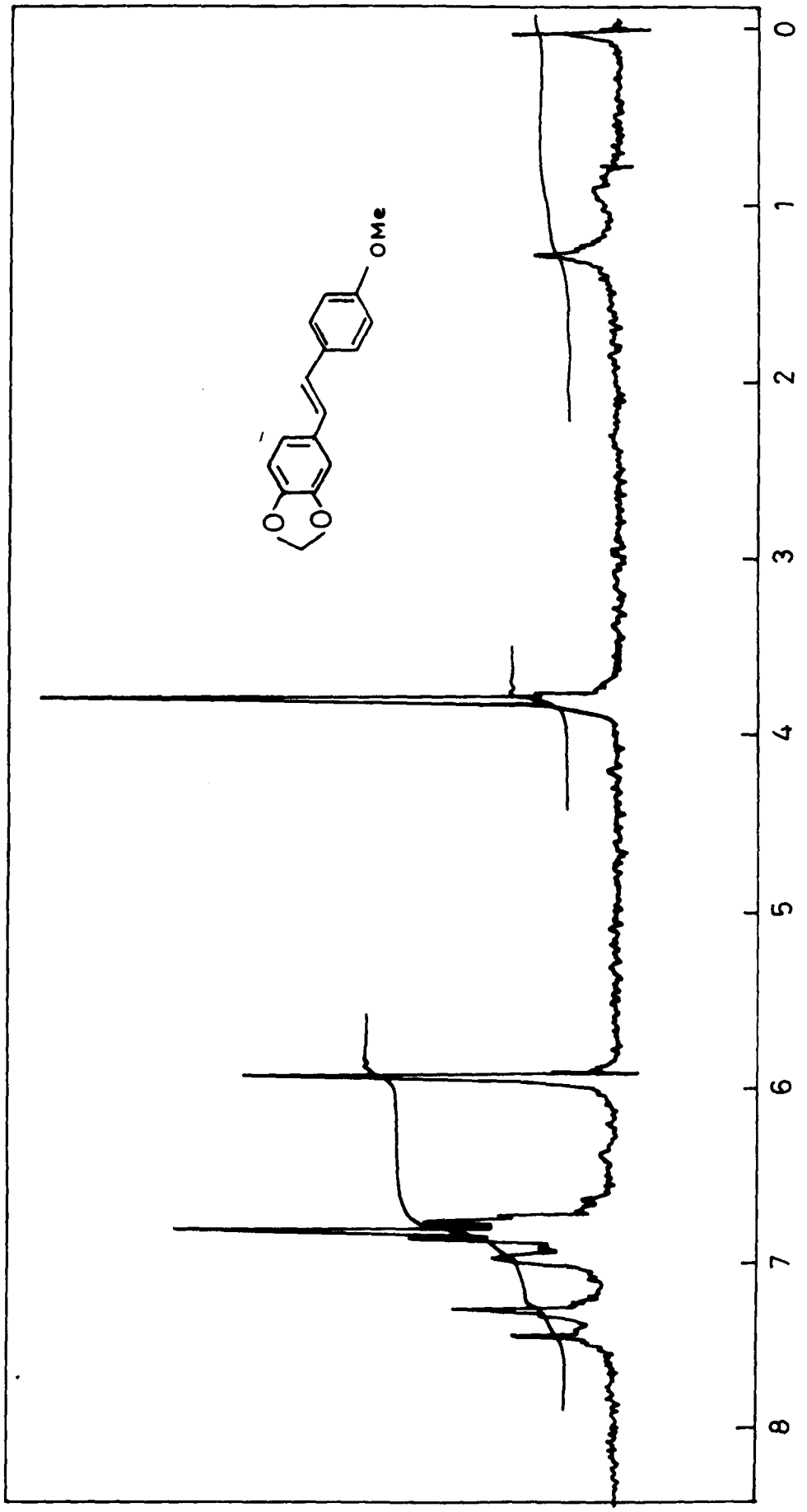



FIG. 8

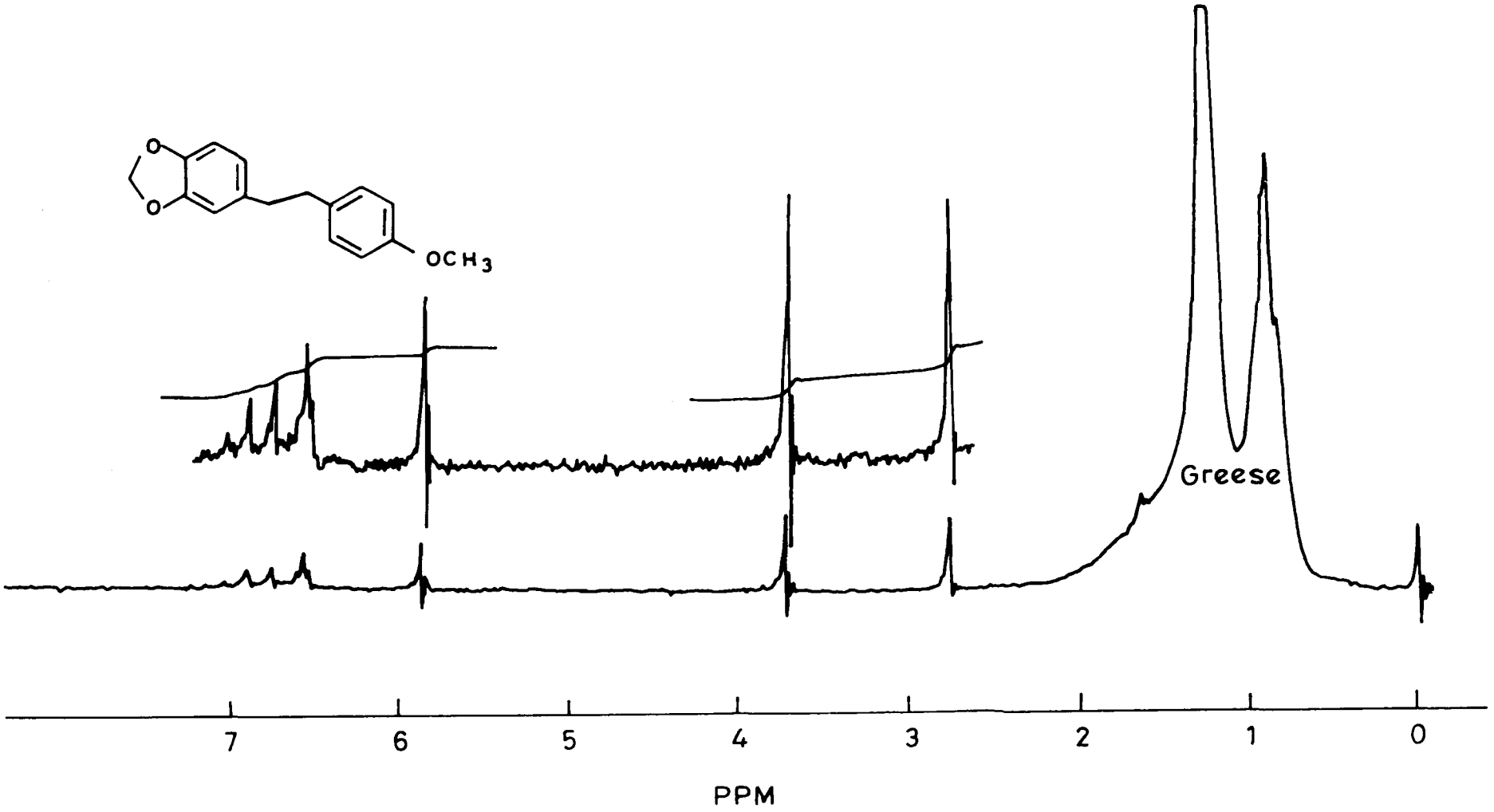
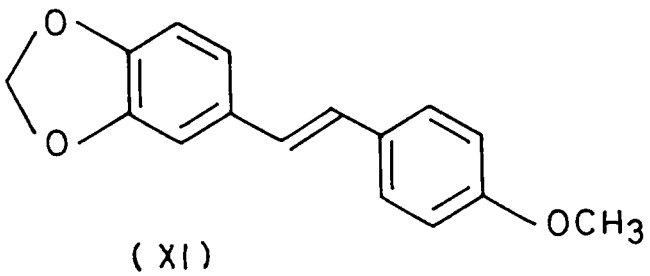



FIG. 9

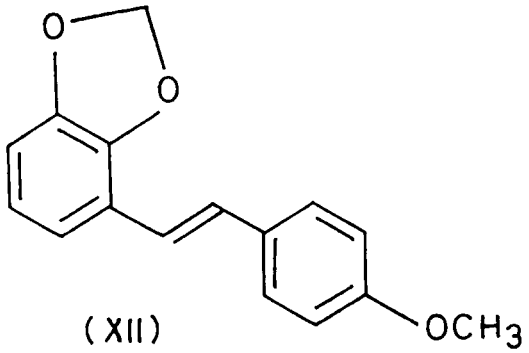
appear as (2H) singlet in the 60 MHz NMR spectrum[12]. While the NMR data shows that one ring is para substituted it leaves the substitution pattern of the other ambiguous, either 2,3 (XII) or 3,4 (XI) methylenedioxy structures being possible. The 60 MHz NMR spectrum of gnetin could not distinguish between the two alternatives (XI) and (XII), the problem was, therefore, solved through synthesis of the biogenetically more likely structure (XI).

Synthesis of 3,4-methylenedioxy-4'-methoxy-trans-stilbene

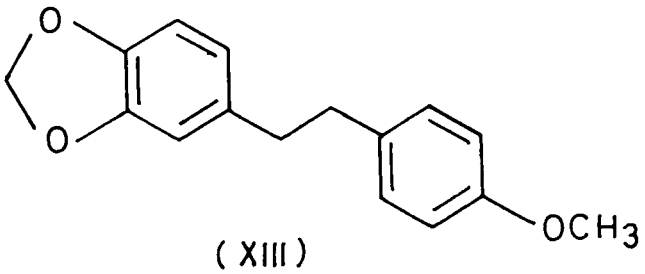
Condensation of 3,4-methylenedioxybenzaldehyde with para-methoxyphenyl acetic acid in the presence of piperidine[9] at 160-170° for about 16 hours gave 3,4-methylenedioxy-4'-methoxy-trans-stilbene as the neutral product. Work up of the acidic fraction gave the corresponding α-carboxylic acid (XIV) which on decarboxylation with $CuCO_3$ in quinoline[10] supplied a further amount of 3,4-methylenedioxy-4'-methoxy-trans-stilbene. The synthetic compound was found identical with the natural sample (Co-TLC, IR, NMR).



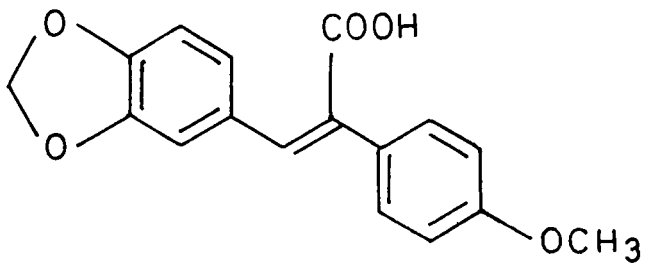

( XI )




( XII )




( XIII )




( XIV )

Oligomeric stilbene

Apart from the compounds so far discussed the plant extract contained a highly polar product also which was eluted when the column was run with pure ethyl acetate. Attempts to crystallise it did not succeed and the crude product was, therefore, acetylated in the usual way. Chromatographic purification of the acetylated material afforded a colourless crystalline solid which had a sharp melting point and gave a single fluorescent spot on TLC plates. The mass spectrum of the solid revealed a molecular weight of 764 which, alongwith combustion data, fits the molecular formula $C_{42}H_{36}O_{14}$. The formation of a hexaacetate is evident from six ketene losses from the molecular ion and the presence of six acetate methyl singlets in the NMR spectrum. The 300 MHz NMR spectrum (Fig. 10) shows further two methoxyls at 3.50 and 3.71 and 11-12 aromatic protons as an unresolved multiplet. The intervening region contains signals of two hydrogens and an overlapping doublet of a doublet making all together 44 protons as against 36 required by the molecular formula. This discrepancy indicates the presence of some impurity, most probably of a fatty acid, since there are a number of losses of 14 mass units in the mass spectrum (Fig. 11). It is all the same certain that the major component of this mixture is

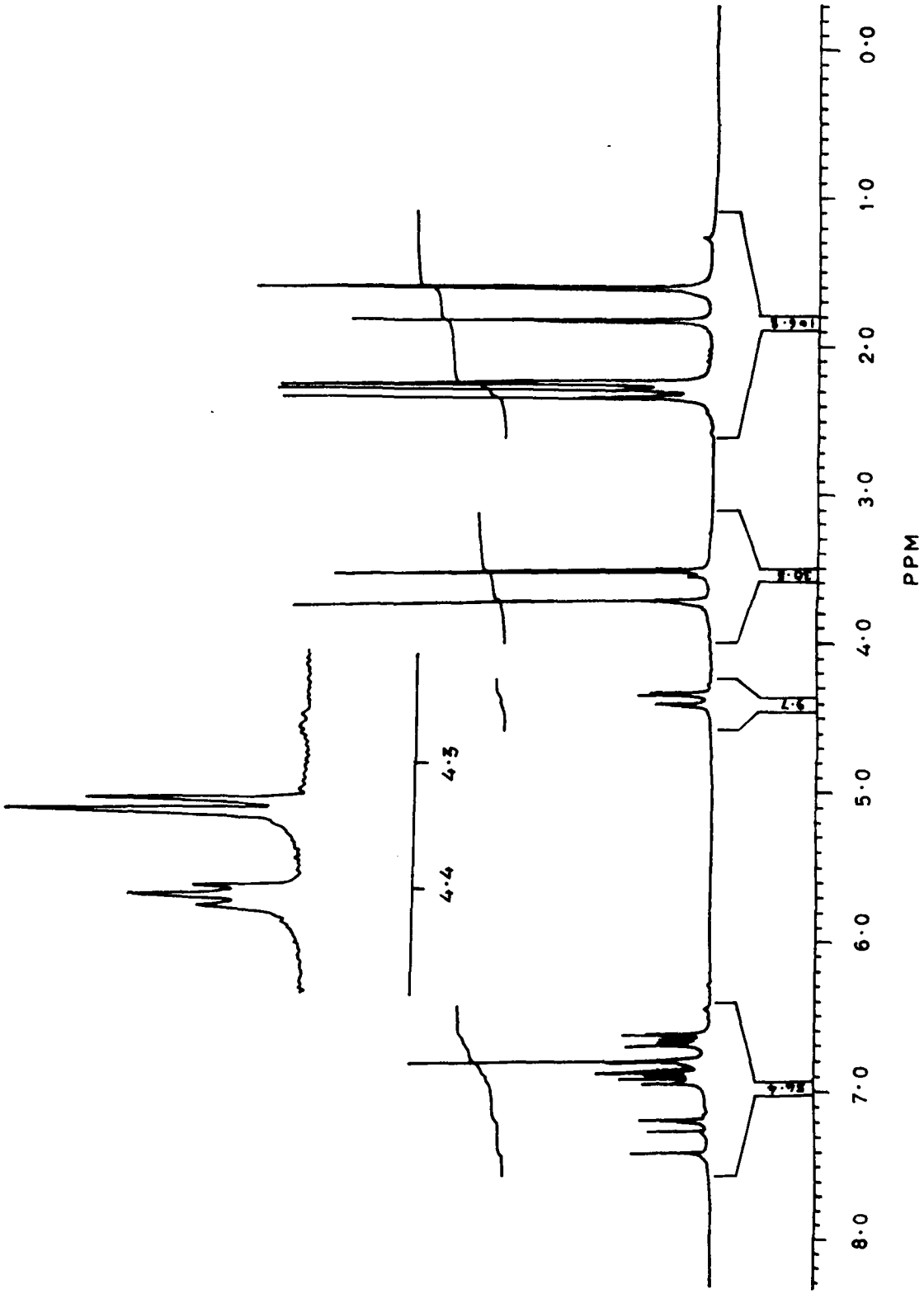
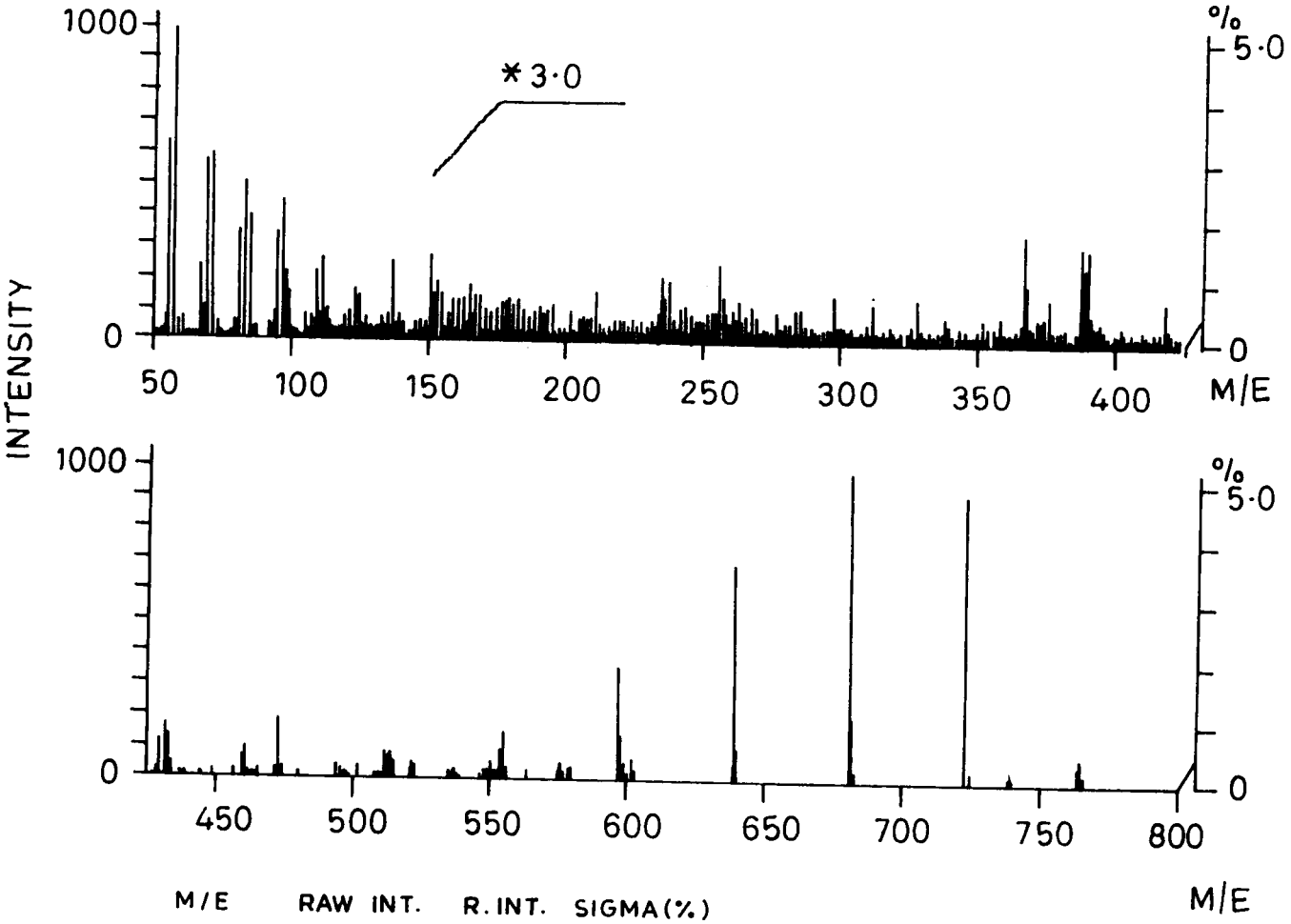



FIG. 10

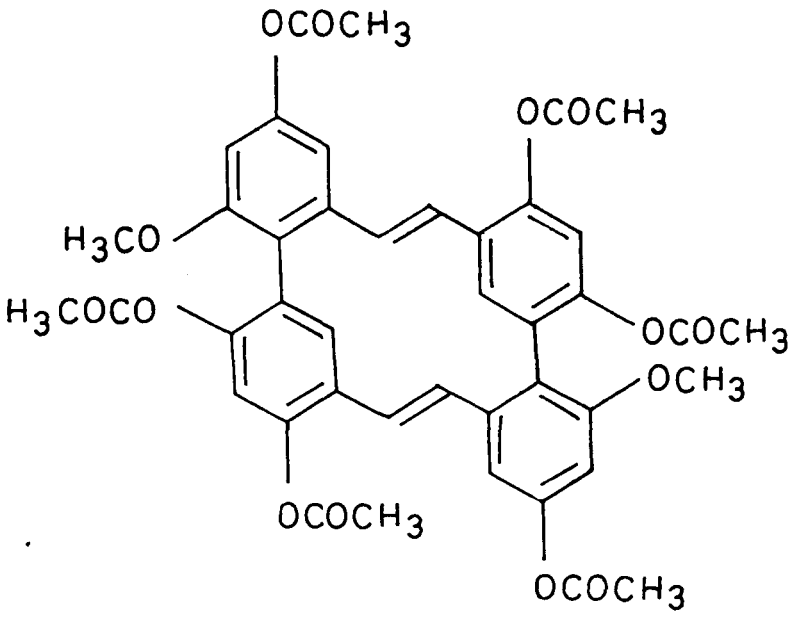



| M/E | RAW INT. | R. INT. | SIGMA(%) | |
|---|---|---|---|---|
| 511·0 | 1·4 | 30·7 | 2·06 | |
| 512·0 | 1·0 | 22·7 | 1·52 | |
| 513·0 | 1·0 | 23·4 | 1·57 | |
| 514·0 | 1·3 | 30·1 | 2·02 | |
| 550·0 | 0·9 | 21·4 | 1·43 | |
| 553·0 | 1·6 | 35·4 | 2·38 | |
| 554·0 | 2·3 | 52·1 | 3·50 | |
| 555·0 | 1·2 | 26·7 | 1·79 | |
| 596·0 | 5·5 | 122·4 | 8·22 | |
| 597·0 | 2·2 | 50·1 | 3·36 | |
| 601·0 | 1·0 | 22·7 | 1·52 | |
| 638·0 | 10·5 | 232·1 | 15·58 | |
| 639·0 | 1·9 | 43·4 | 2·92 | |
| 680·0 | 15·5 | 341·1 | 22·91 | |
| 681·0 | 3·3 | 74·2 | 4·98 | |
| 722·0 | 14·1 | 309·6 | 20·79 | |
| 763·0 | 1·0 | 22·7 | 1·52 | |
| 764·0 | 1·2 | 27·4 | 1 84 | END |

FIG. 11

a dimeric stilbene with six acetate and two methoxyl functions. Assuming oxidative cyclisation one can formulate a working structure (XV) which has the required molecular formula, functionalities and the number of deshielded protons. The singlet at 1.59 alongwith the doublet and a double doublet would then have to be assigned to a fatty acid impurity of the type $CH_3(CH_2)_n$-CH—CH-COOH. Attempts to purify the product

either by preparative TLC or repeated crystallisation did not succeed and exposure to alkali led to destructive hydrolysis. It is likely that one has here in hand, as in the case of the xanthone of Calophyllum wightianum, a clathrate[13]. The problem is proposed to be taken up again when some more plant material is available.



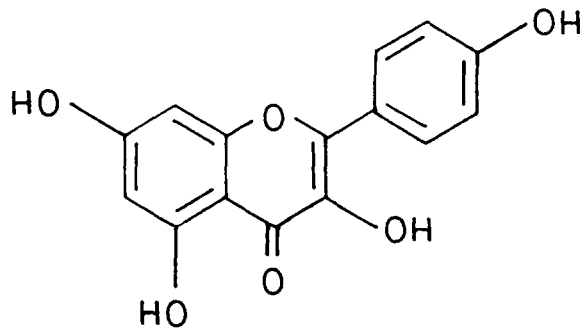

( XV )

Maytenus emarginata (syn. Maytenus molina; Celastraceae)

Maytenus species have been the subject of extensive investigation in the United States of America and China. Initial reports of the presence of antitumor and antileukemic compounds, collectively known as maytansinoids, were published by Morris Kupchan's group[14-18]. Between 1980 and 1984 Chinese workers published over twenty papers on maytansinoids[19-21] but since all of these are in Chinese journals not much use could be made of this literature in designing an appropriate extraction and work up procedure for Maytenus emarginata. One of the best methods to isolate maytansinoids has been published by H.H.S. Fong et al.[22] Maytenus emarginata is used for medicinal purposes in South India and unconfirmed news paper reports credit it with having antileukemic activity.

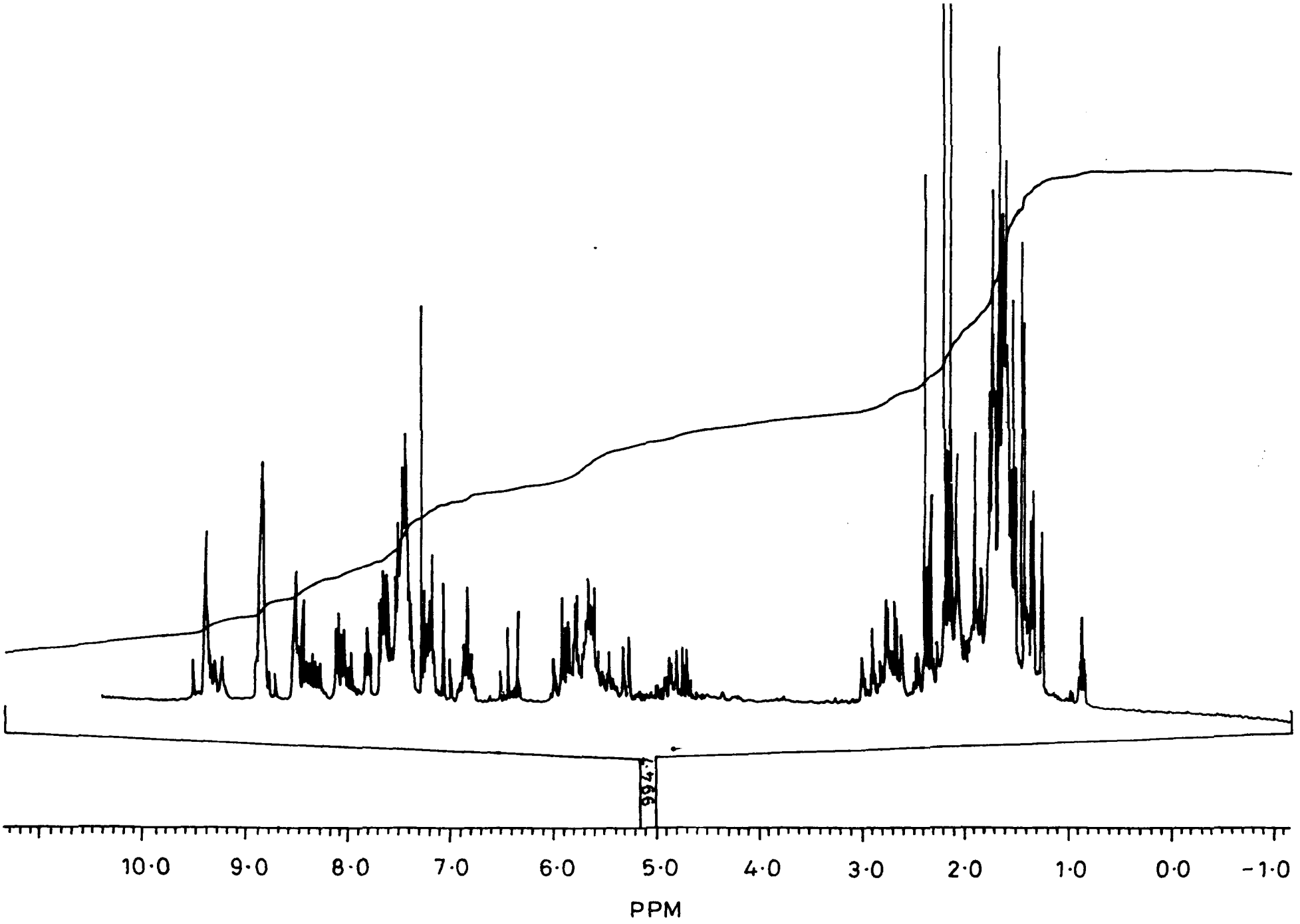
Maytenus emarginata was collected from Hyderabad (South India) where it grows wild in rocky regions. Preliminary tests indicated that the leaves and roots contain alkaloids whereas stem wood does not. Maytenus emarginata leaves were extracted with benzene and then with methanol. Alkaloids were found to be present in the methanol extract only. The residue obtained on removal of methanol was treated with 5% HCl and the aqueous acidic portion was exhaustively

extracted with ethyl acetate. The free bases were precipitated by addition of sodium bicarbonate to the aqueous fraction. The ethyl acetate layer was worked up separately to give a poly-oxygenated flavone, readily identified as kaempferol (XVI)[23] from its spectral data. TLC of the crude alkaloid showed it




( XVI )

to be a mixture of atleast four components. Chromatographic separation proved extremely tedious and supplied only one product, MA-1, which did not show any resolution on high performance TLC plates and appeared to be mostly comprised of a single entity on spectroscopic examination. Another product, MA-2, also appeared to be pure on TLC but the complexity of its 300 MHz NMR spectrum (Fig. 12) suggests that it is a mixture. HPLC has been used extensively in the


994·7
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0·0
-1·0
PPM

separation of maytansinoids but the facility was not available locally and application of this method had to be deferred.

MA-1

The UV spectrum of MA-1 (Fig. 13) shows absorption maxima at 223 and 280 nm which indicates the presence of a trans cinnamoyl moiety[24]. The IR spectrum (Fig. 14) shows a carbonyl band at 1645 $cm^{-1}$ which, taken together with the UV absorption, establishes the presence of a Ph-CH=CH-C(=O)-N- moiety.

The mass spectrum, $M^{+\cdot}$ at m/z 405, provides the molecular formula $C_{25}H_{31}N_3O_2$ which immediately suggested that MA-1 is celacinnine (XVII)[25] which has been reported from three other Maytenus species[25-27]. The 90 MHz NMR spectrum (Fig. 15) of MA-1 shows the characteristic doublets of the trans cinnamoyl moiety at 6.80 and 7.71 (J=15.5 Hz)[25]. The region between the two doublets contains the unresolved multiplet of ten aromatic protons and similar unresolved broad multiplets are present between 1.3-2.2, 2.2-2.8, 2.8-3.8 and 3.8-4.0. The broad signal from 3.85-4.0 integrates for 1 proton and corresponds in value to the chemical shift of the C-8 hydrogen of celacinnine[25]. The total rise of integral between 1.3-4.0 is equivalent to the presence of 19 hydrogens as required for

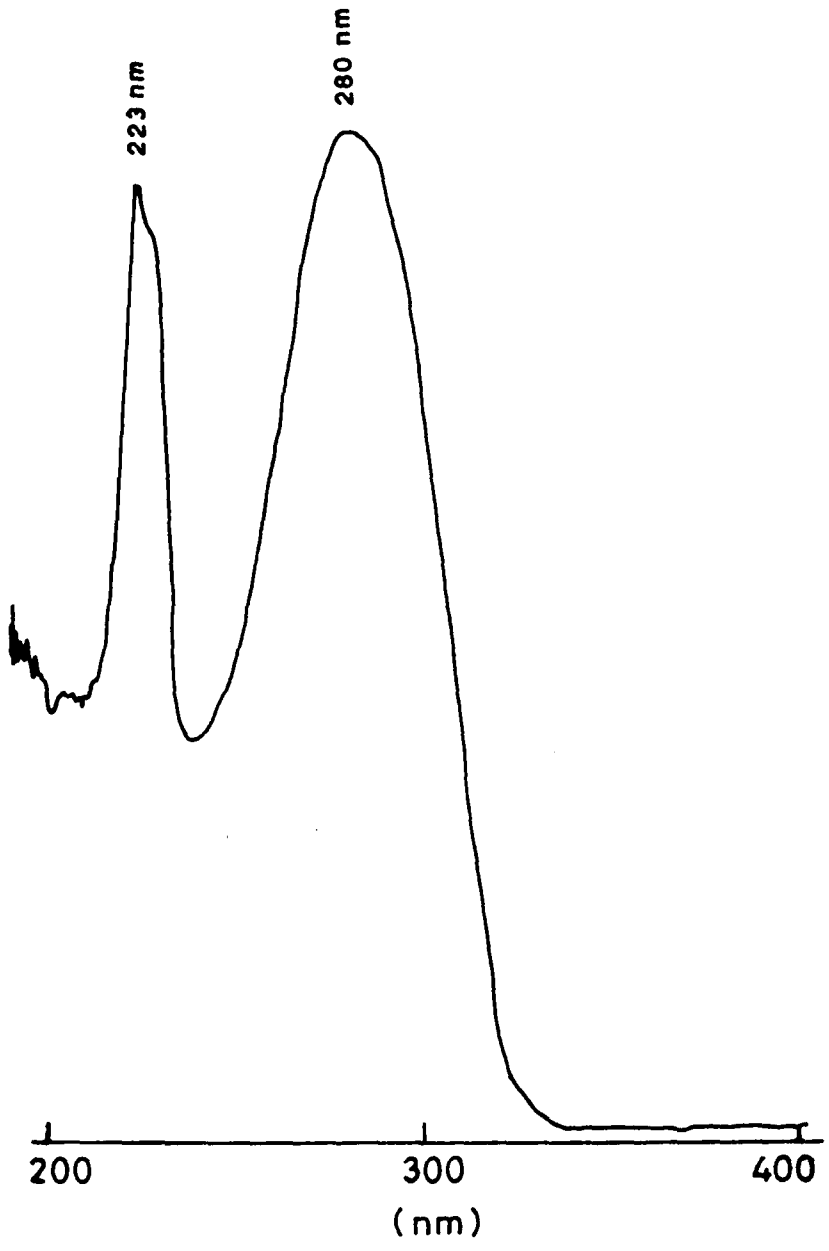



FIG. 13

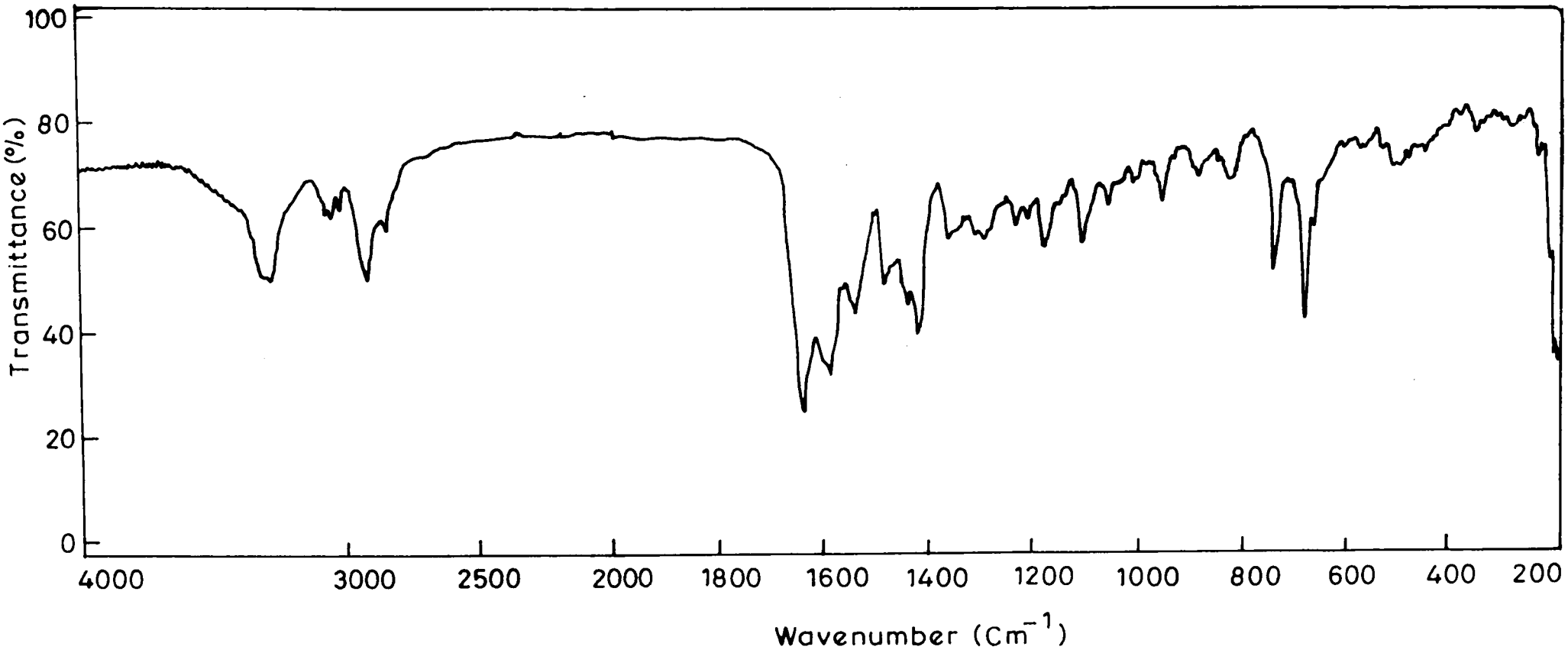

100
80
60
40
20
0
Transmittance (%)
4000
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
200
Wavenumber ($Cm^{-1}$)

FIG. 14

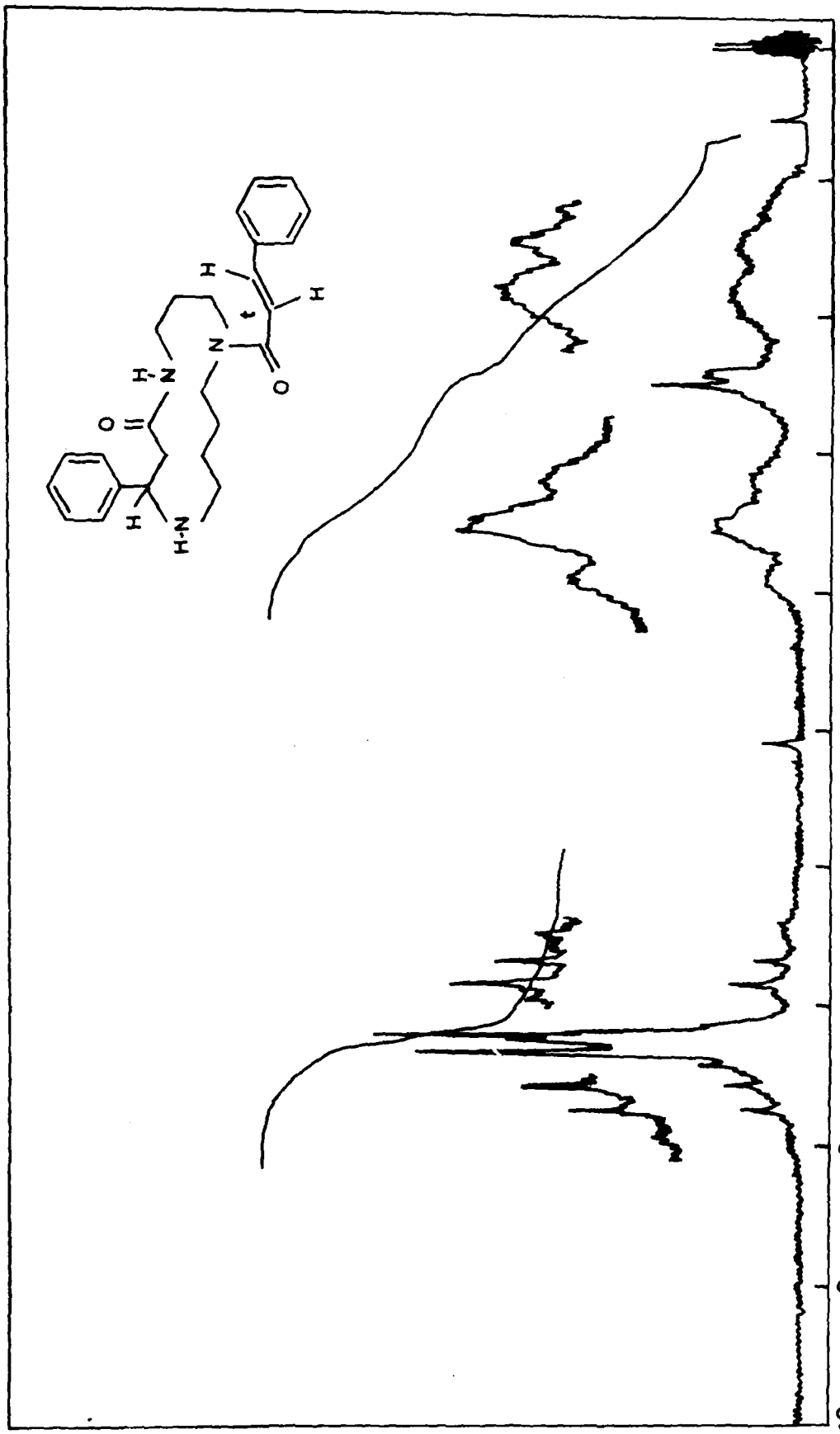
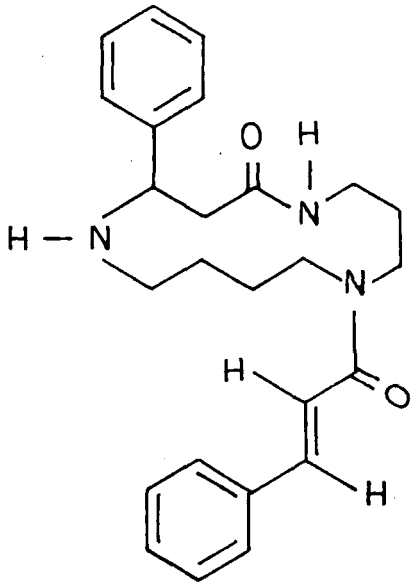



FIG. 15

celacinnine. The discrepancy with celacinnine is the difference in melting points of the two i.e. 152° for MA-1 and 203° for celacinnine. MA-1, however, could not be obtained in a crystalline form even after repeated crystallisations.



( XVII )

The assumption that MA-1 is slightly impure celacinnine is supported by the 300 MHz NMR spectrum (Fig. 16) which shows some other interesting features as well. The most significant difference from 90 MHz NMR spectrum is the splitting of the α-hydrogen of the cinnamoyl group to a double doublet (J=15.3, 5.5 Hz). This long range coupling to one of

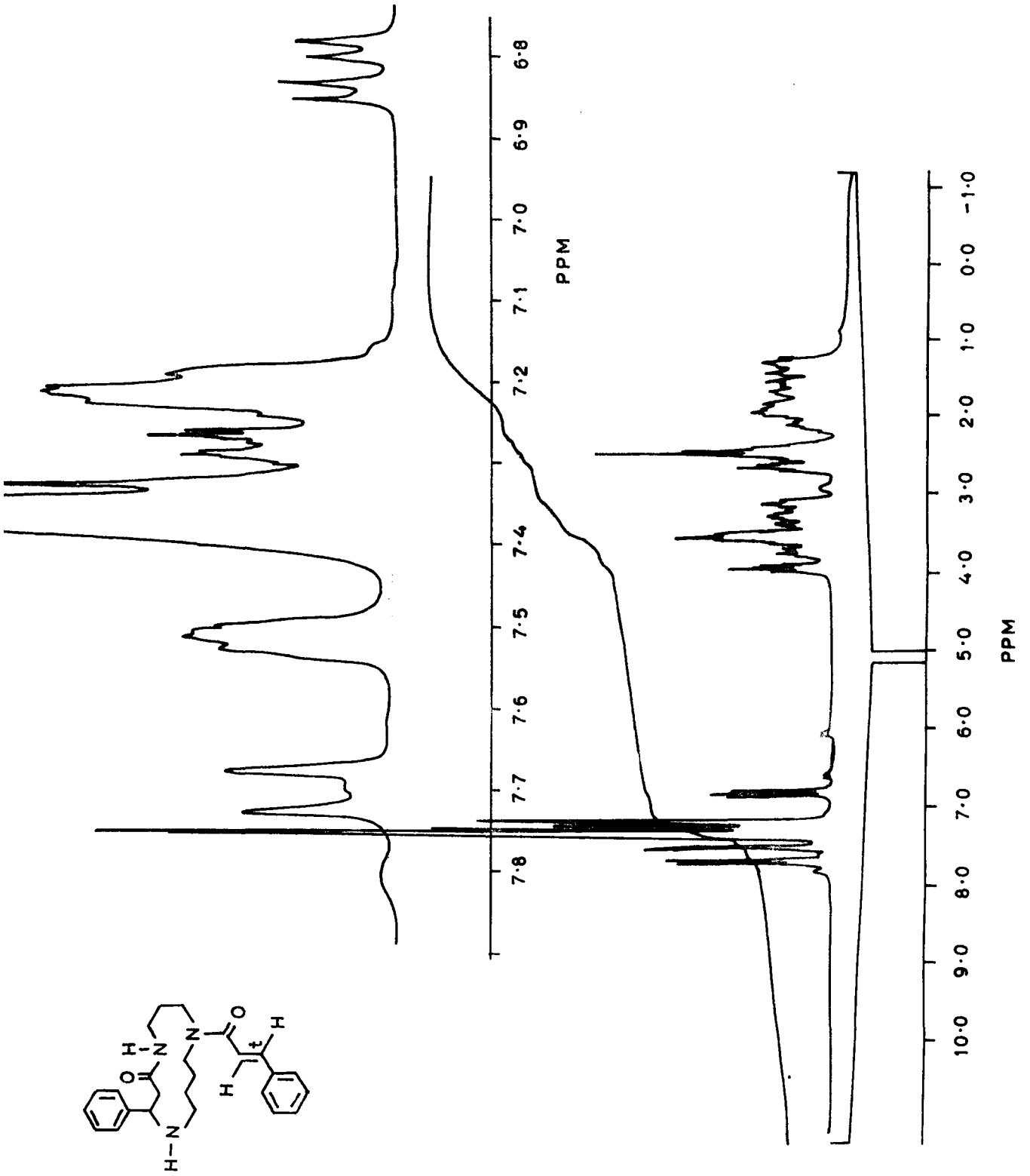
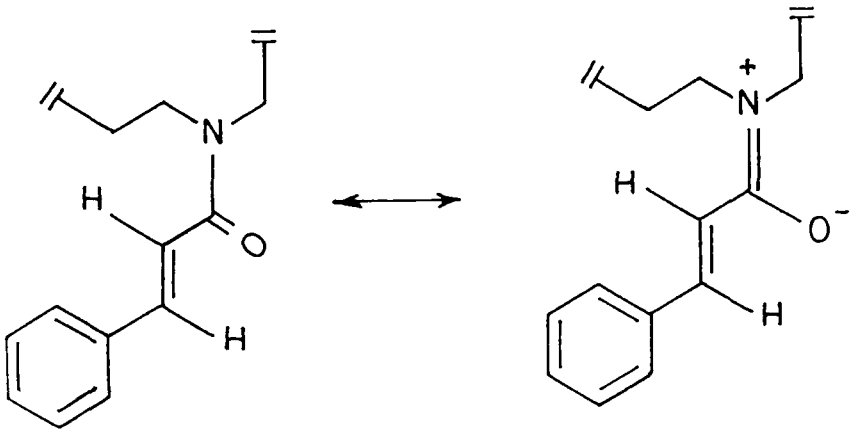

PPM
6·8
6·9
7·0
7·1
7·2
7·4
7·5
7·6
7·7
7·8
PPM
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0·0
-1·0

FIG. 16

the methylene hydrogens adjacent to the amide nitrogen is suggestive of considerable double bond character for the intervening C-N bond (XIX) i.e. a substantial resonace contribution of part structure (XVIII). The signals of the



( XVIII )  ( XIX )

protons of the macrocyclic ring are not resolved in this spectrum also but that of the C-8 hydrogen is more clearly marked out. It does not, however, appear as the clear triplet reported by Kupchan *et al.*[25] in the 100 MHz NMR spectrum, though the chemical shift, 3.992 is very close to the reported value, 4.0. This may be due to slow equilibration of the different conformations of the macrocyclic nucleus at room temperature, better resolved spectrum should result at higher

temperature. Significant from the stand point of the purity of MA-1, however, are two small quartets at 6.05 and 6.61. The presence of these suggests that the impurity in celacinnine is that of celallocinnine[25], the other cis isomer, the chemical shifts of α- and β-protons of which have the same value.

The mass spectrum of MA-1 also corresponds closely to that of celacinnine showing strong peaks at m/z 405, 274, 160, 145 and 131 as reported.

Triterpene lactone-A

Extraction of Maytenus emarginata roots afforded, alongwith β-amyrone (XX) and β-amyrin (XXI), a high melting compound designated as lactone-A. The UV spectrum of the compound was featureless but IR evidence (Fig. 17) pointed to the possible presence of a 5-membered lactone moiety and one or more hydroxyl groups. The high resolution mass spectrum gave the accurate molecular weight 454.3445 which fits the molecular formula $C_{30}H_{46}O_3$ (required 454.3446). The $M^{+\cdot}$ is extremely unstable and its breakdown gives rise to the most stable fragment having the accurate mass 246.1614 corresponding to the molecular formula $C_{16}H_{22}O_2$ (required 246.1602).

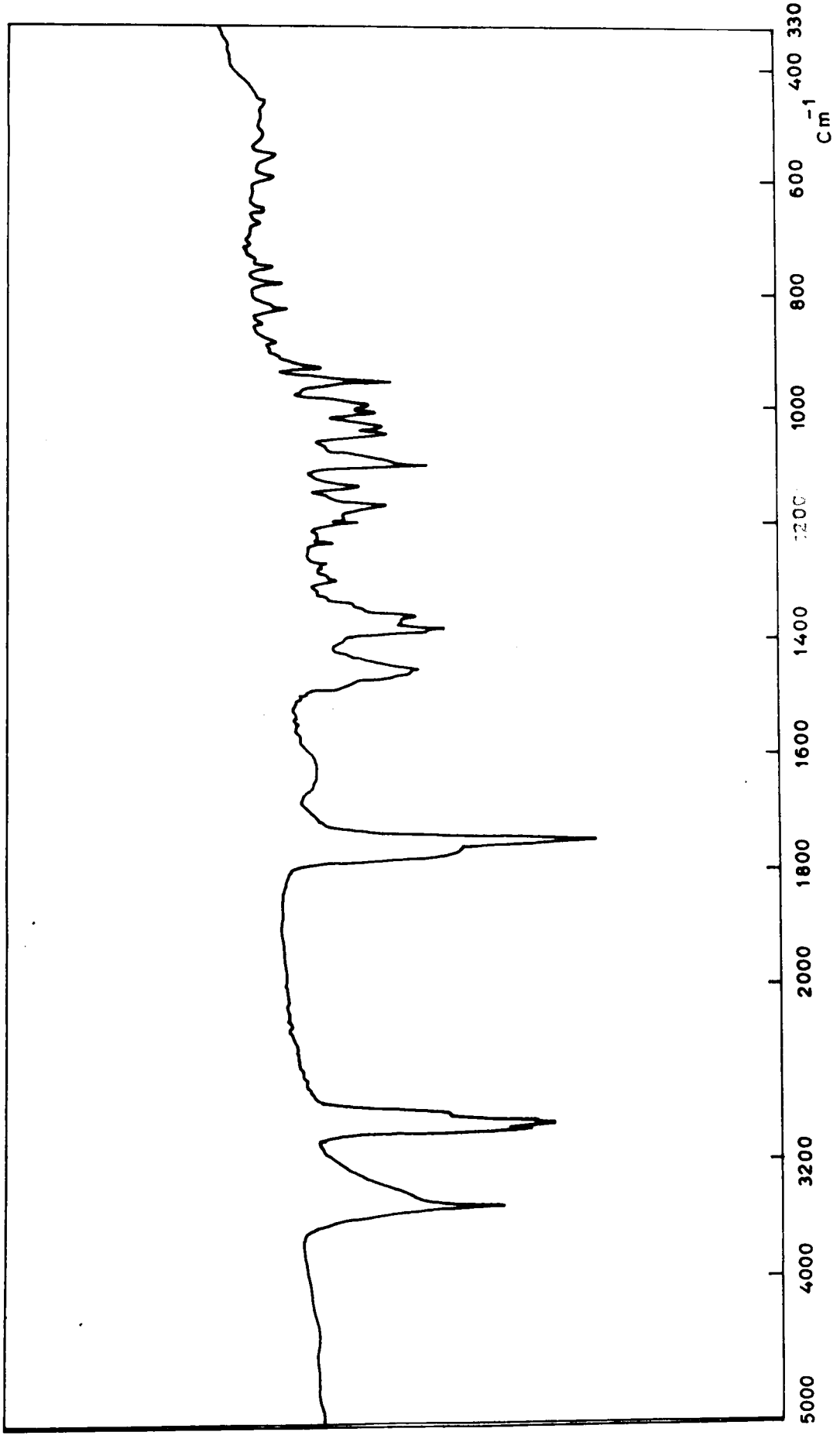



FIG. 17

Subtraction of this from $C_{30}H_{46}O_3$ gives the formula of the other fragment of the retro-Diels-Alder cleavage as shown in scheme III.

The β-amyrin nucleus suggested by the mass spectral data is supported by the 300 MHz NMR spectrum (Fig. 18) which has singlets due to seven methyls and a multiplet at 3.22 agreeing with the signal of the C-3 C<u>H</u>-OH grouping. The olefinic hydrogen appears at the standard value 5.29 as a clear triplet[28] which leaves only the doublet at 4.13 (J=5.4 Hz) to be assigned. Combination of the data leads to two possible structures (XXII) and (XXIII). The hydrogen under the lactone oxygen of (XXII) is known to give a doublet rather than the expected triplet because of the effect of severe strain on the bond angles of ring E[29]. A similar situation should exist in the case of (XXIII) also which was isolated from <u>Tripterygium wilfordii</u> (Celastraceae) by Chinese workers[30] and the melting point they report (322-326°) is close to that of lactone-A (328-330°). As against this the reported melting point of (XXII) is 240-243°. Further the carbonyl band of (XXII) is at 1776 $cm^{-1}$ whereas of lactone-A is at 1750 $cm^{-1}$. Lactone-A is, therefore, (XXIII). A comparative sample or the reprint of the paper by the Chinese workers could not so far be obtained.

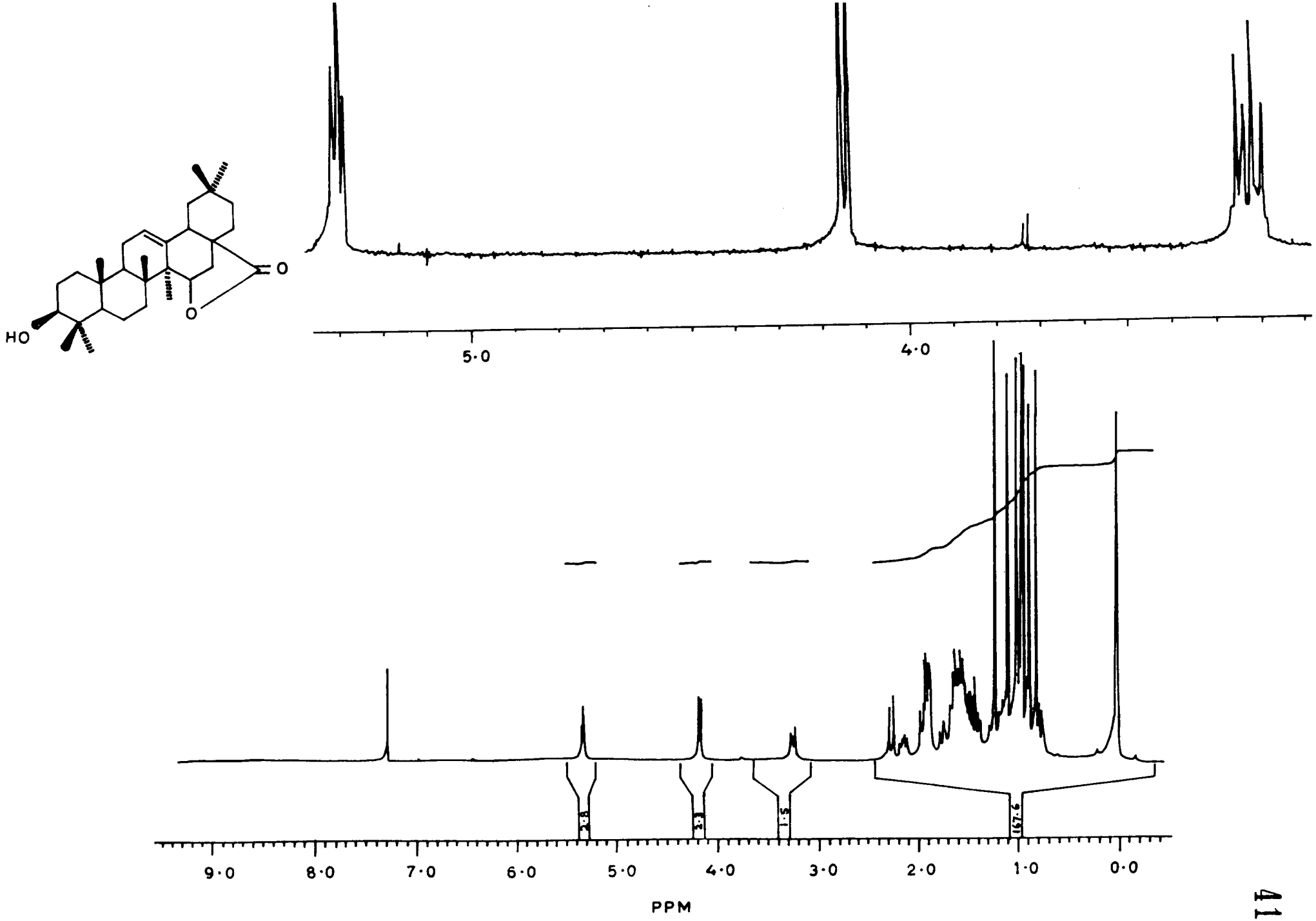
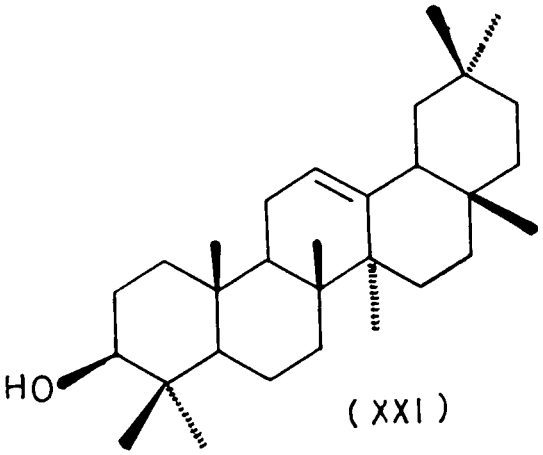
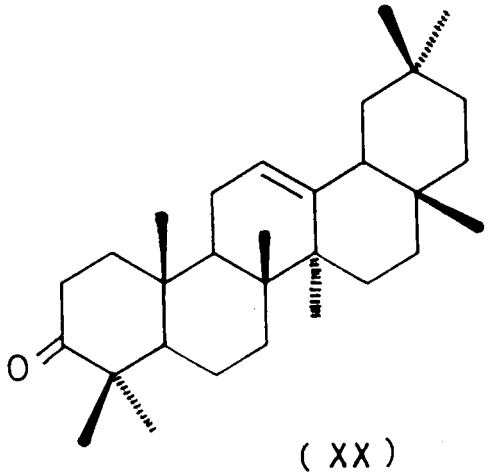


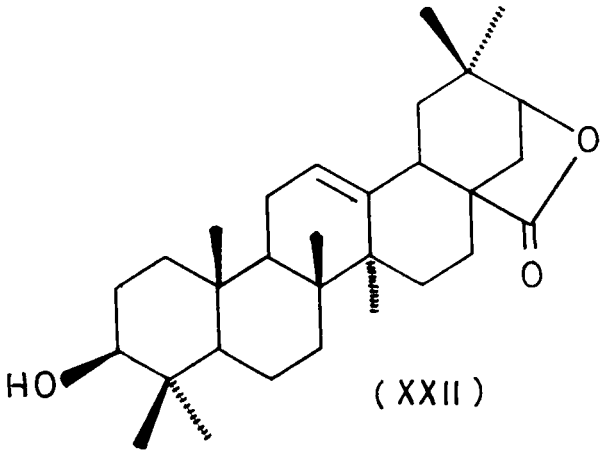

FIG. 18



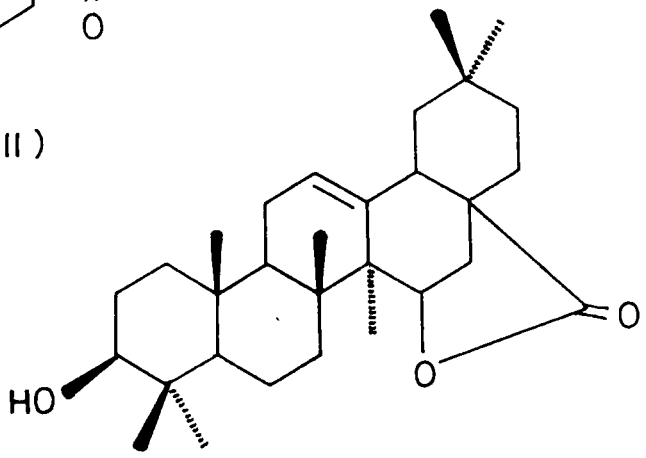

( XXI )



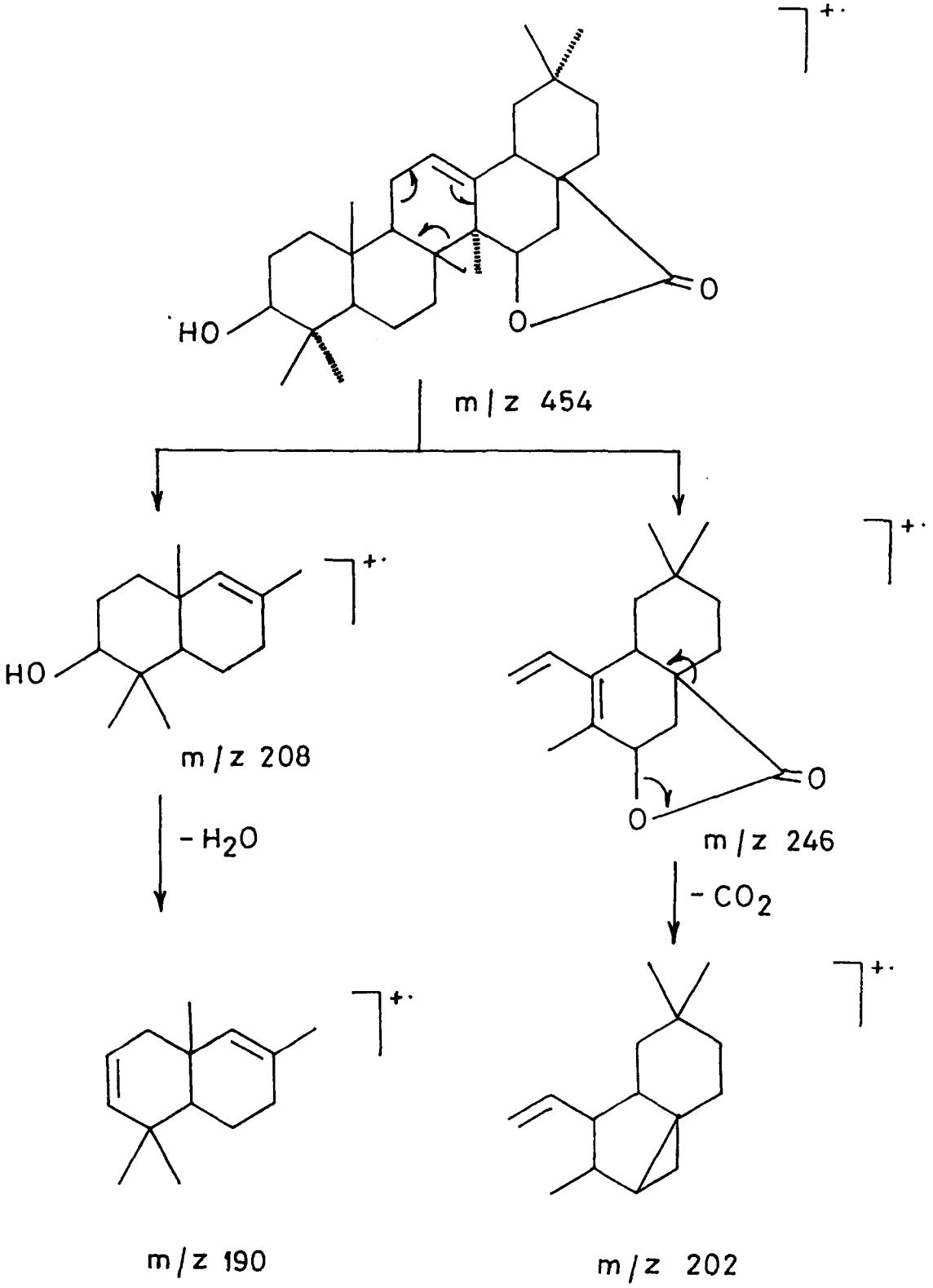

( XX )




( XXII )




( XXIII )

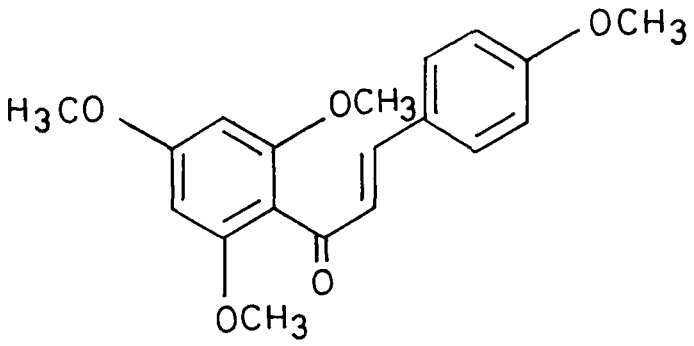



scheme III

Excoecharia agallocha (Euphorbiaceae)

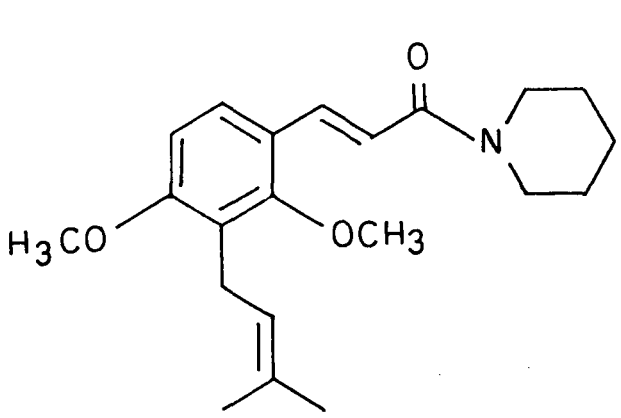
The plant Excoecharia agallocha was collected, alongwith some other medicinal plants, during a tour of Goa (West India) because of several medicinal uses ascribed to its acrid latex in the Wealth of India[31]. The tree is also listed among those considered poisonous to stock and is used as a fish poison.

The petroleum ether extract of the plant was found to contain a solid, readily identified as 2',4',6',4-tetramethoxychalcone (XXIV) on the basis of spectral evidence and comparison with a synthetic sample and an oil positive to Dragendorff reagent. The mass spectrum of the oil shows $M^{+\cdot}$ at m/z 343 corresponding to the molecular formula $C_{21}H_{29}NO_3$.

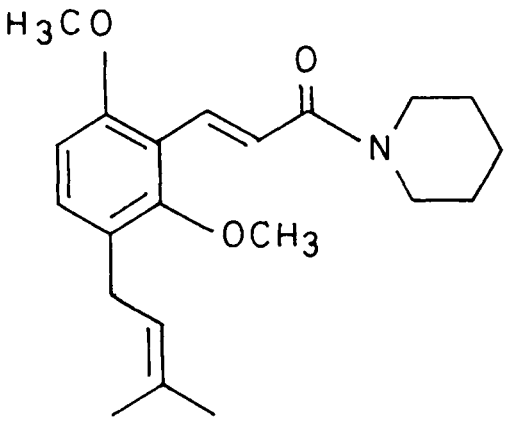



( XXIV )

The UV spectrum indicates that it is a cinnamic acid derivative and IR carbonyl at 1650 $cm^{-1}$ definitely identifies the derivative as an amide[32]. The 60 MHz NMR spectrum (Fig. 19) confirms these conclusions and establishes the presence of piperidine and a γ,γ-dimethylallyl moiety[33,34]. Taken together with the presence of two methoxyl singlets in the NMR spectrum the data permits only structures (XXV) and (XXVI). Differentiation between these two by spectroscopic methods is difficult but (XXV) appeared biogenetically more likely and was, therefore, synthesised[35].




(XXV)




(XXVI)

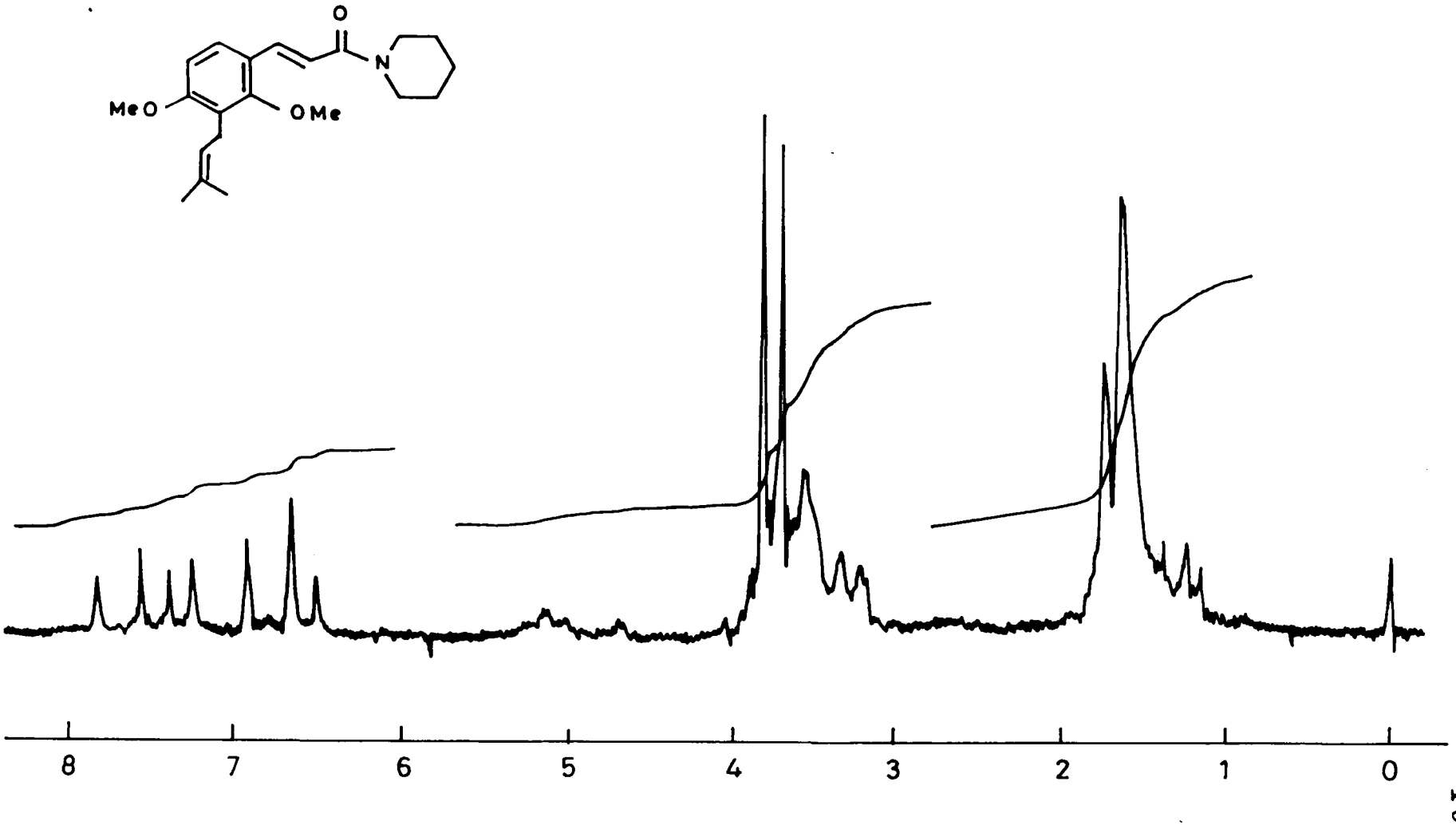
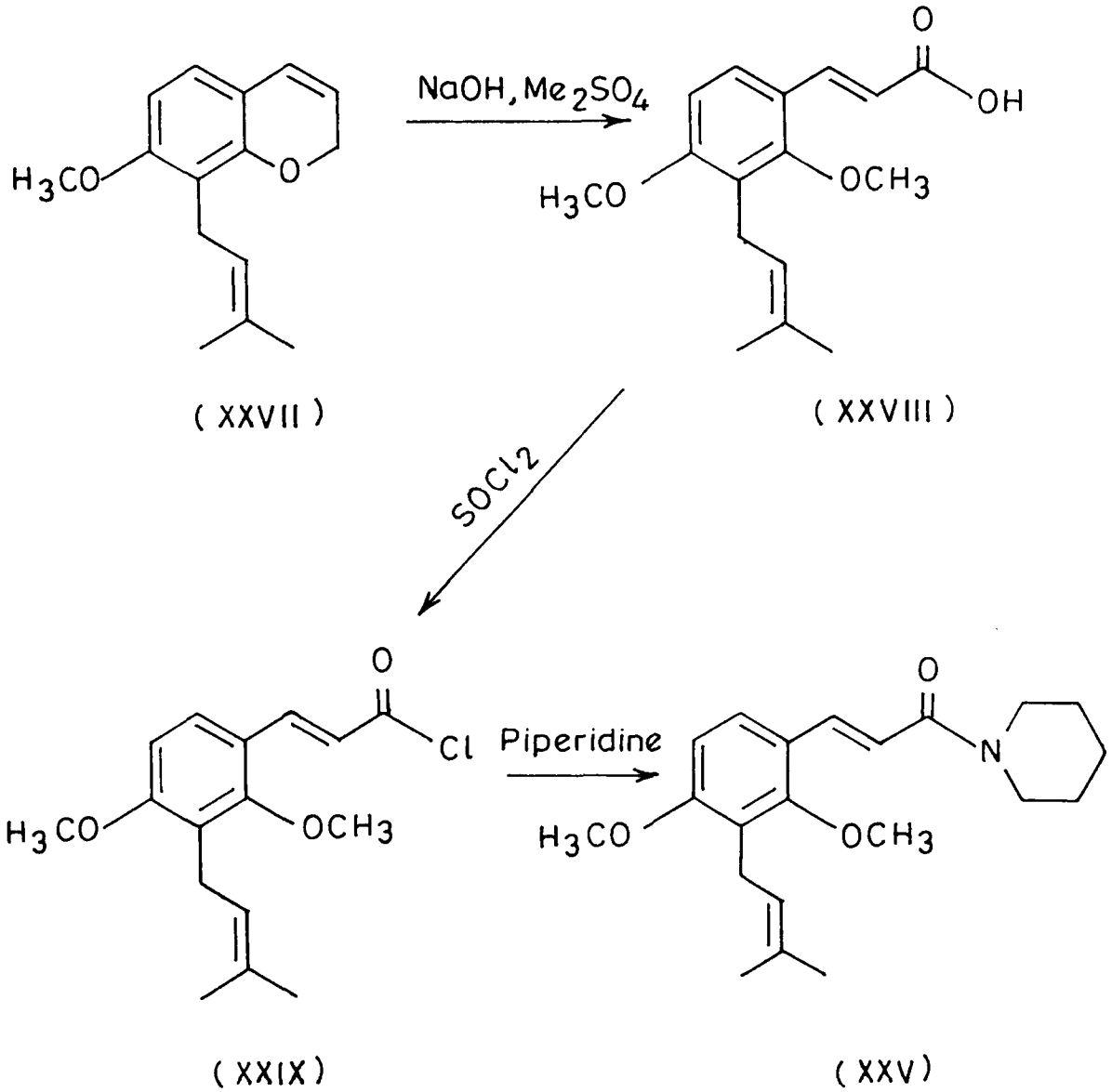



FIG. 19

Methylative ring opening of osthol[36] (XXVII) gave the cinnamic acid (XXVIII) and conversion of this to chloride (XXIX) followed by treatment with piperidine gave a product (XXV) which by Co-TLC, IR and NMR was found identical with the isolated compound (scheme IV).

It is interesting to note that such piperidine alkaloids have so far been encountered in Piperaceae only and this is the first example, to our knowledge, of isolation of such a compound from a Euphorbiaceae plant.




( XXVII )

( XXVIII )

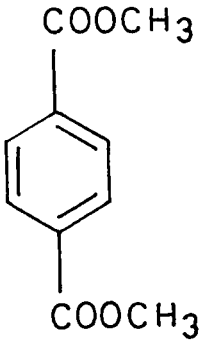
( XXIX )

( XXV )

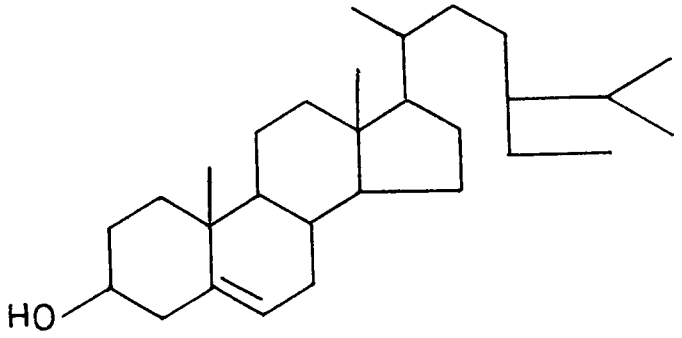
scheme IV

Cissus pallida (syn. Vitis pallida; Vitaceae)

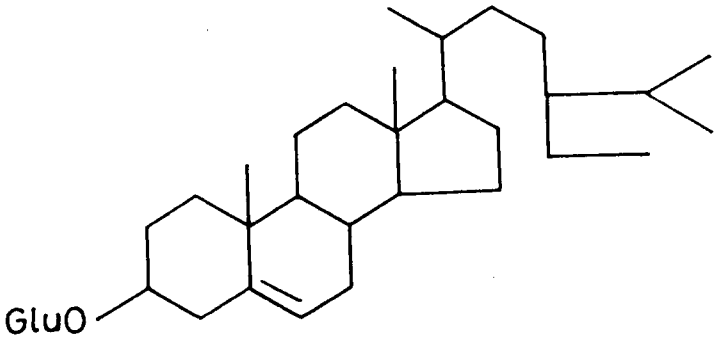
Cissus pallida is a woody perennial shrub found in the Garhwal region of Himalayas in North India and in the mountain range that runs parallel to the west coast of India. According to 'Wealth of India' bruised roots of this plant are effective in the treatment of rheumatic swellings[37]. Isolation of stilbenes[38], tetracyclic triterpenoids[39] and alkaloids[40] has been reported from other species but a survey of literature indicated that Cissus pallida has not been so far studied. Members of the family Vitaceae have considerable medicinal value and their chemical investigation has yielded phytoalexins and antimicrobial compounds. Accordingly when it came to our knowledge that the plant Cissus pallida also grows abundantly in the hilly outskirts of Hyderabad, South India, it was included for collection during a tour of that region. The fresh plant material was identified by the Botany Department, Osmania University, Hyderabad. The alcohol extract of the leaves gave a positive test for alkaloids, the stem wood, however, contained only non-basic components which were isolated through column chromatography of the ethyl acetate extract. These were labelled as CP-1, CP-2, CP-3, CP-4 and CP-5. CP-2, CP-3, CP-4 and CP-5 were identified as dimethyl terephthalate (XXX), β-sitosterol (XXXI), β-sitosterol



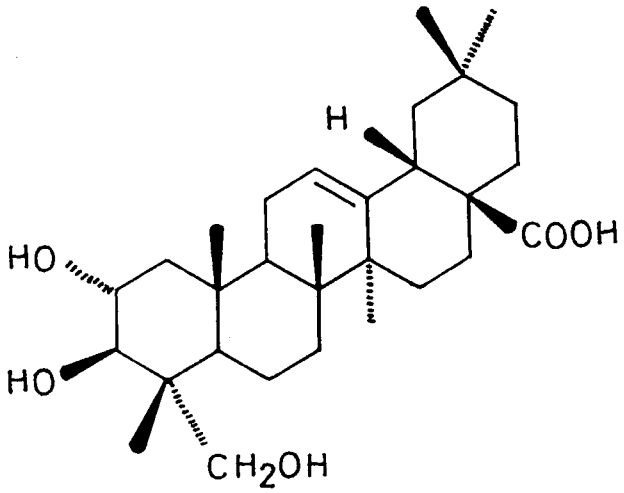

( XXX )




( XXXI )




( XXXII )



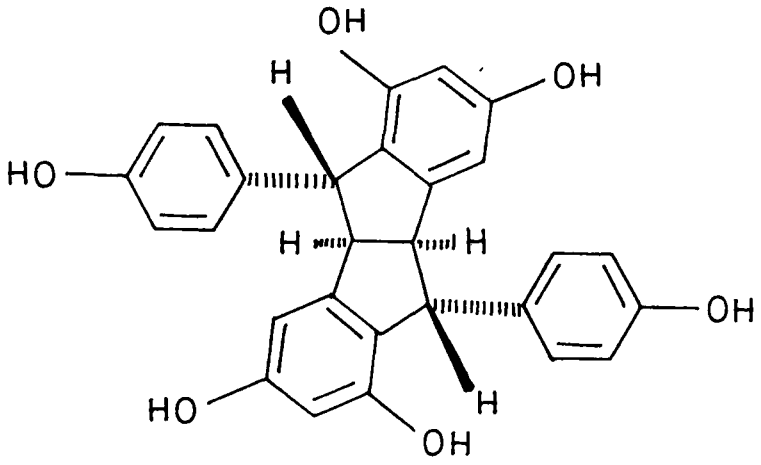

( XXXIII )

glucoside (XXXII)[41] and arjunolic acid (XXXIII)[42].

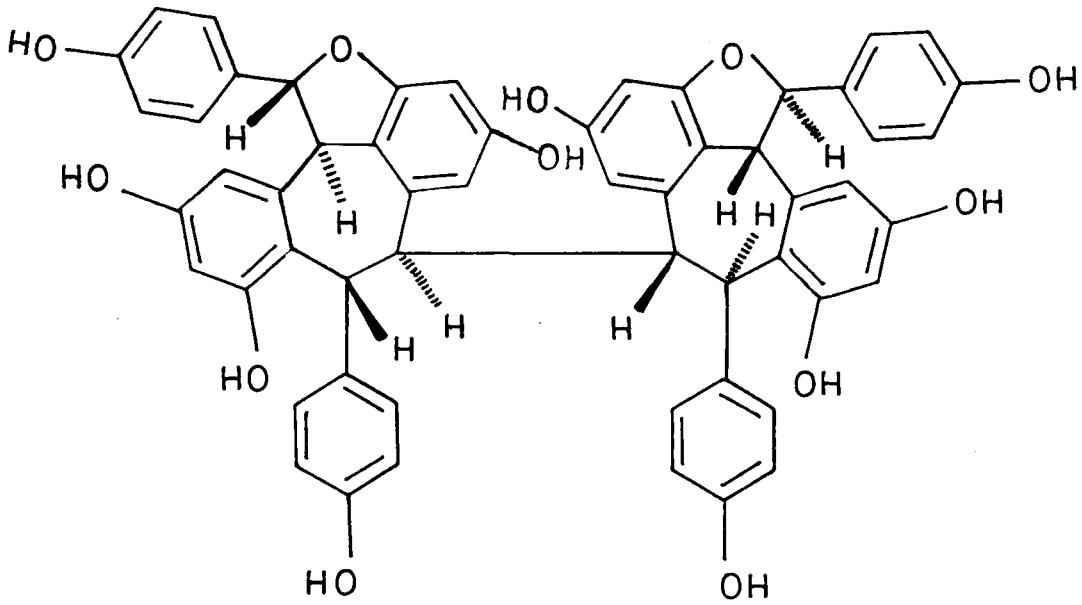
The physical and spectral characteristics of CP-1 did not agree with those of any known compound and structure (XXXIV), which shows it to be a stilbene dimer, is proposed for it on the basis of its UV, IR, NMR and mass spectra; it is supported also by plant taxonomic and biogenetic considerations. Pallidol (XXXIV)[43] adds one more new compound to the relatively small class of oligomeric stilbenes. It is of interest in this context that the first compound of this type, hopeaphenol, a resveratrol tetramer was isolated only in 1965 from Hopea odorata and Balanocarpus heimii[44]. Hopeaphenol was later isolated from Shorea robusta and Shorea telura[45]. A number of oligomeric stilbenes have been isolated since 1977 from plants belonging to the families Dipterocarpaceae, Gnetaceae and Vitaceae. While their structures show wide variation in the carbocyclic framework they are all formed through oxidative cyclisation of two or more resveratrol units. The oligomeric stilbenes were reviewed[46] in 1980 but some more have been isolated since then. The presently known ones are given in table 1. It may, however, be noted that stilbene oligomers, alongwith the monomer resveratrol, reported from Vitis vinifera (Vitaceae) were isolated from fungal infected or UV irradiated plants i,e. are phytoalexins or abnormal metabolites.

Table 1

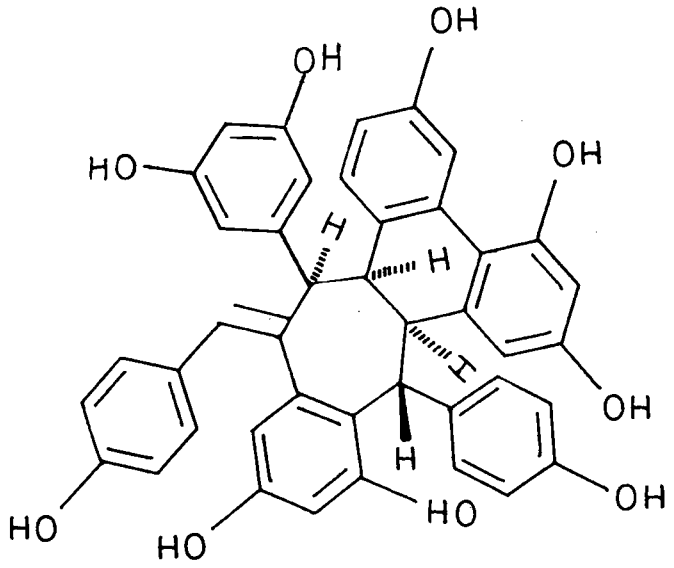
| Trivial name | | | Source | Family |
|---|---|---|---|---|
| Hopeaphenol | (XXXV), | resveratrol tetramer | Hopea odorata | Dipterocarpaceae[47] |
| Stemonoporol | (XXXVI), | resveratrol trimer | Stemonoporus affinis | Dipterocarpaceae[48] |
| Copalliferol-A | (XXXVII), | resveratrol trimer | Vateria copallifera | Dipterocarpaceae[49,50] |
| Vaticaffinol | (XXXVIII), | resveratrol tetramer | Vatica affinis | Dipterocarpaceae[51,52] |
| ε-Viniferin | (XXXIX), | resveratrol dimer | Vitis vinifera | Vitaceae[53,54] |
| α-Viniferin | (XL), | resveratrol trimer | Vitis vinifera | Vitaceae[55] |
| Gnetin-A | (XLI), | resveratrol dimer | Gnetum leyboldii | Gnetaceae[56] |
| Gnetin-B | (XLII), | resveratrol dimer | Gnetum leyboldii | Gnetaceae[57] |
| Gnetin-E | (XLIII), | resveratrol trimer | Gnetum leyboldii | Gnetaceae[57] |



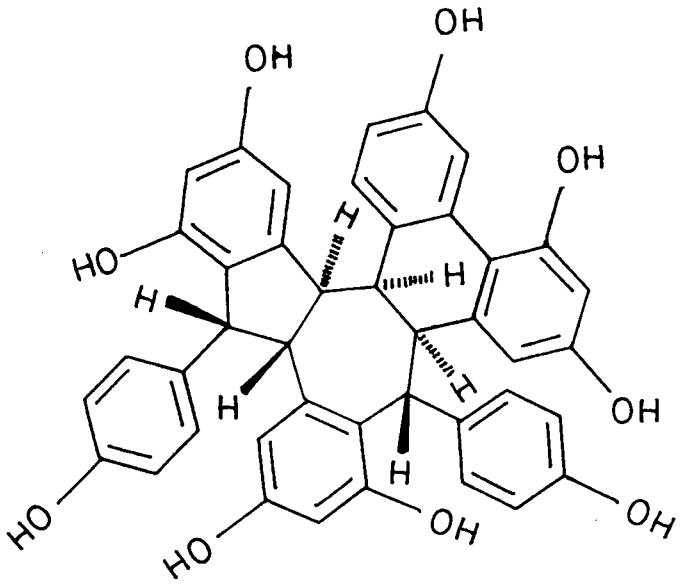

( XXXIV )



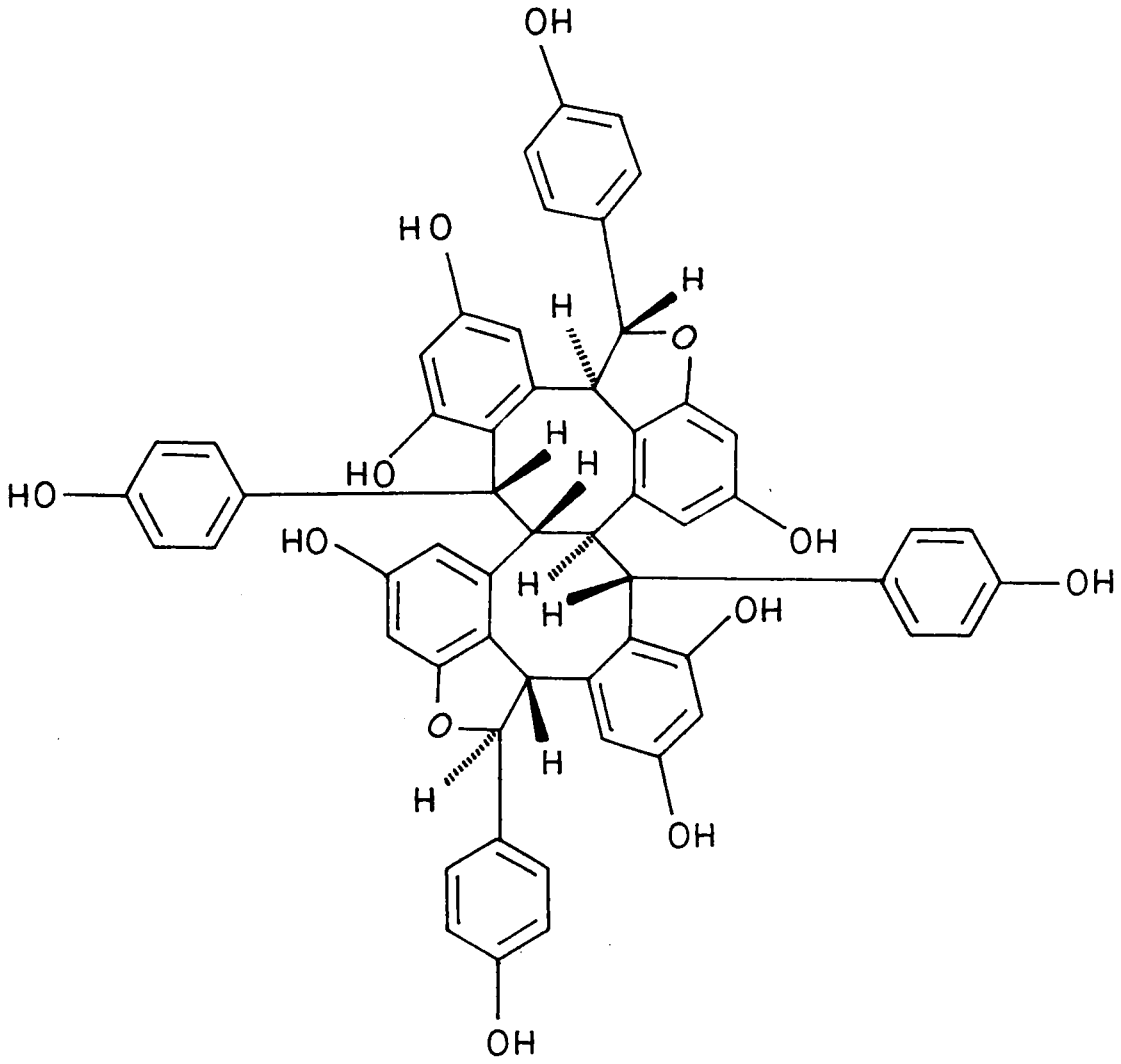

( XXXV )



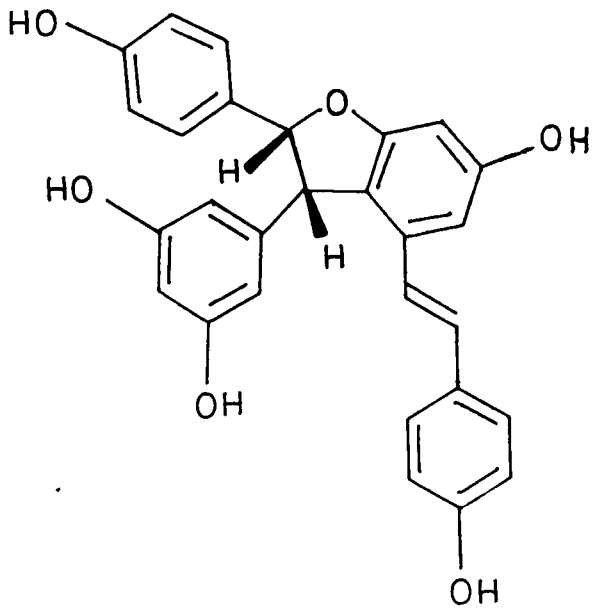

( XXXVI )



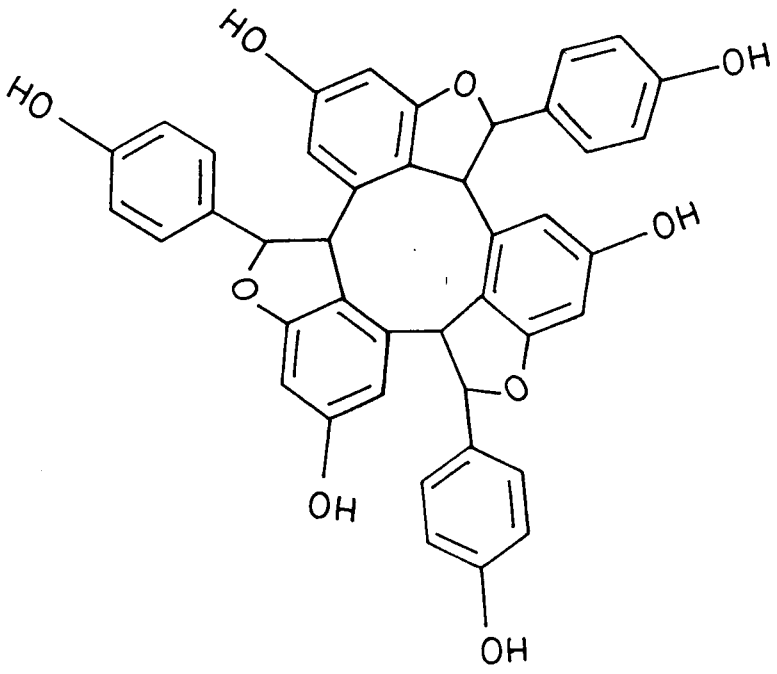

( XXXVII )



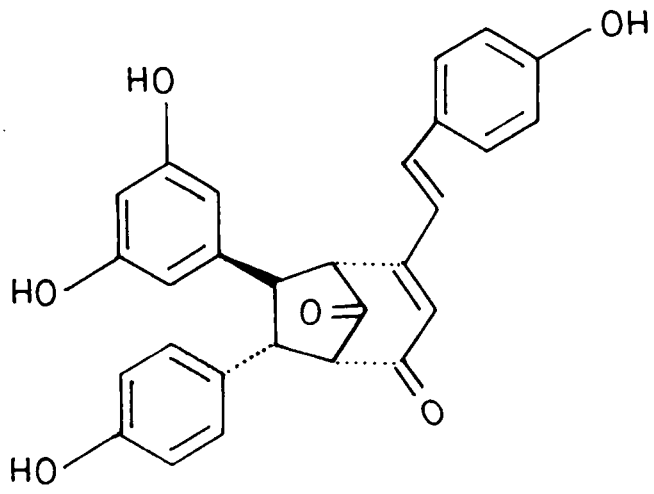

( XXXVIII )



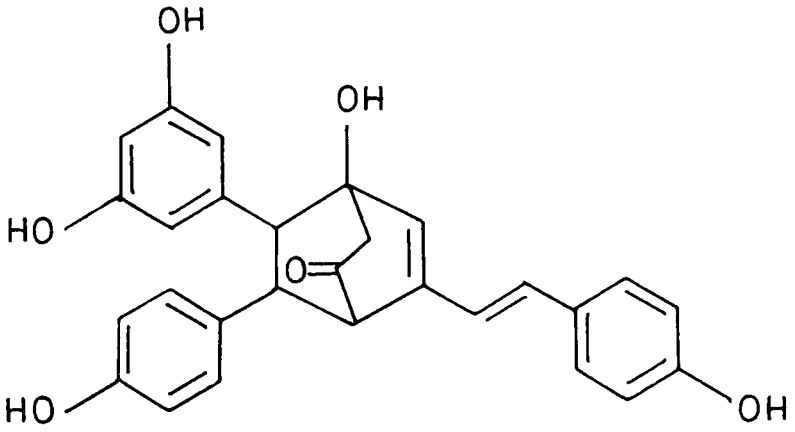

( XXXIX )



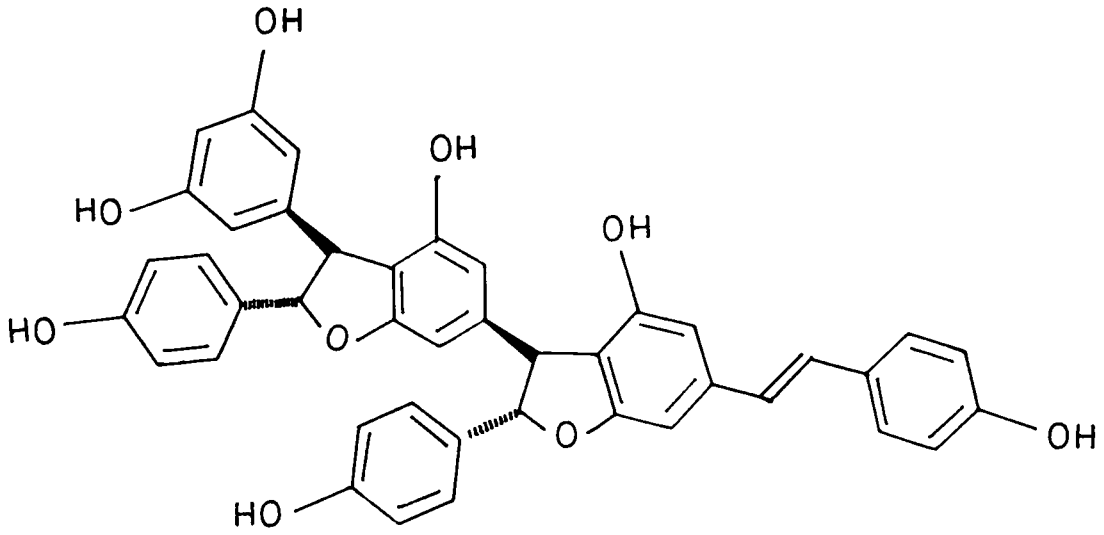

( XL )




( XLI )




( XLII )




( XLIII )

Pallidol was eluted from the column with benzene-ethyl acetate (50:50). Repeated attempts at crystallisation did not succeed but its homogeneity was evident from TLC examination and was confirmed through crystallisation of the acetate and methyl ether. It may be noted here that polyhydroxystilbene oligomers are usually obtained as amorphous powder[49]. The high resolution mass spectrum of the compound showed $M^{+\bullet}$ at m/z 454.14022 which agrees with the molecular formula $C_{28}H_{22}O_6$ (required 454.1412). The IR spectrum (Fig. 20) of pallidol shows no carbonyl absorption but has a prominent hydroxyl band at 3350 $cm^{-1}$. Aromatic absorption at 1605 $cm^{-1}$ coupled with the unusually strong band at 835 $cm^{-1}$ is suggestive of the presence of a 1,4-disubstituted benzene ring[49,58]. Absence of conjugation is also apparent from the UV spectrum (Fig. 21) which shows only phenolic absorption at 284 nm and has no maxima above 300 nm. Pallidol did not give any distinct colour with ferric chloride and absence of vicinal dihydroxy grouping was confirmed by measuring the UV absorption after addition of sodium acetate-boric acid.

Owing to its complete insolubility in $CDCl_3$ the NMR spectrum of pallidol was run in DMSO-$d_6$. The 100 MHz NMR spectrum (Fig. 22) initially obtained is remarkable for the simplicity of the aromatic region for a compound which

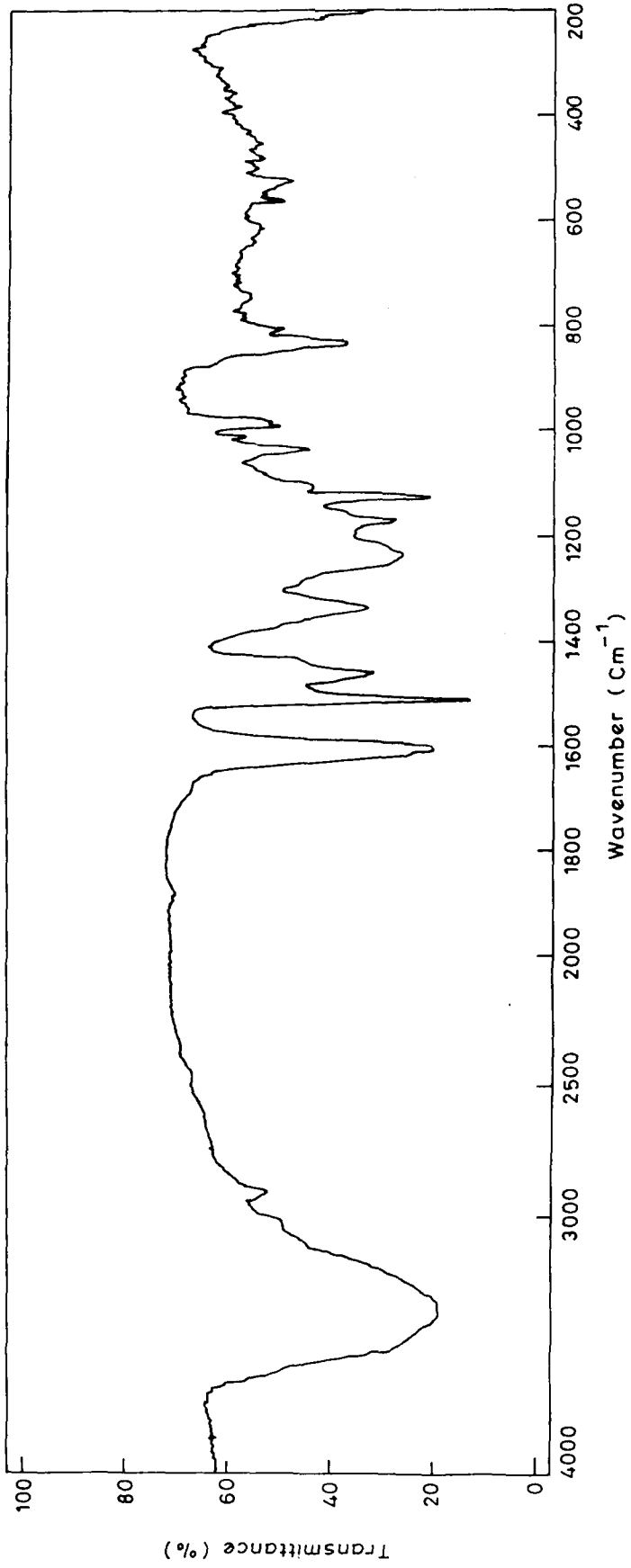

Transmittance (%)
100
80
60
40
20
0
4000
3000
2500
2000
1800
1600
1400
1200
1000
800
600
400
200
Wavenumber ( $Cm^{-1}$ )

FIG. 20

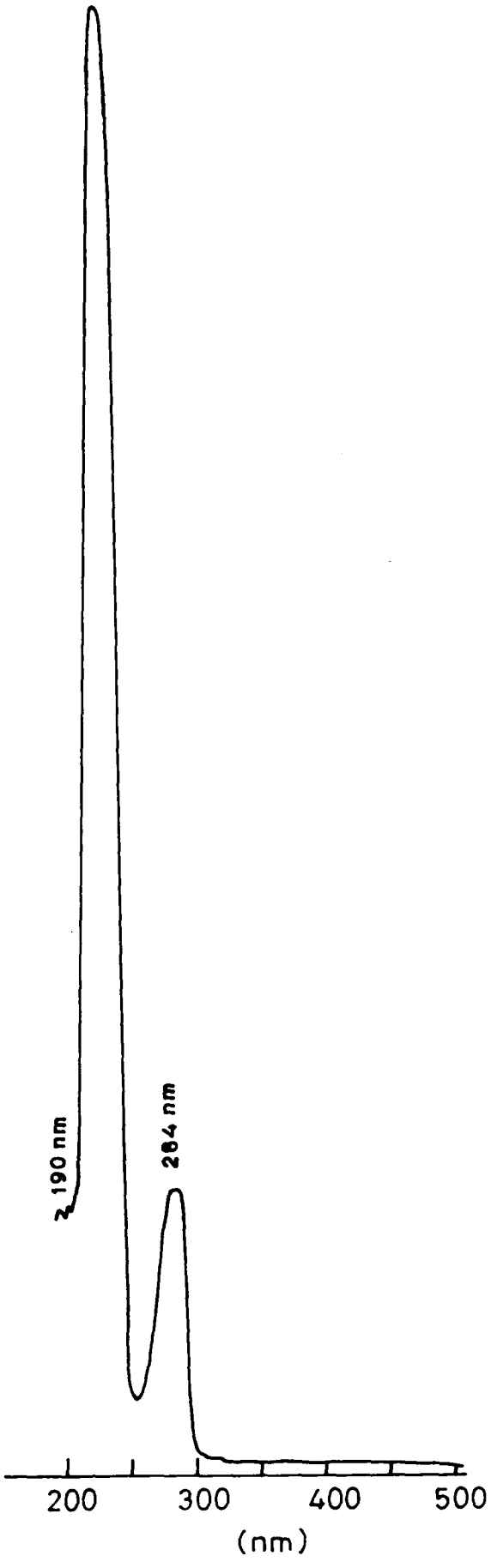



FIG. 21

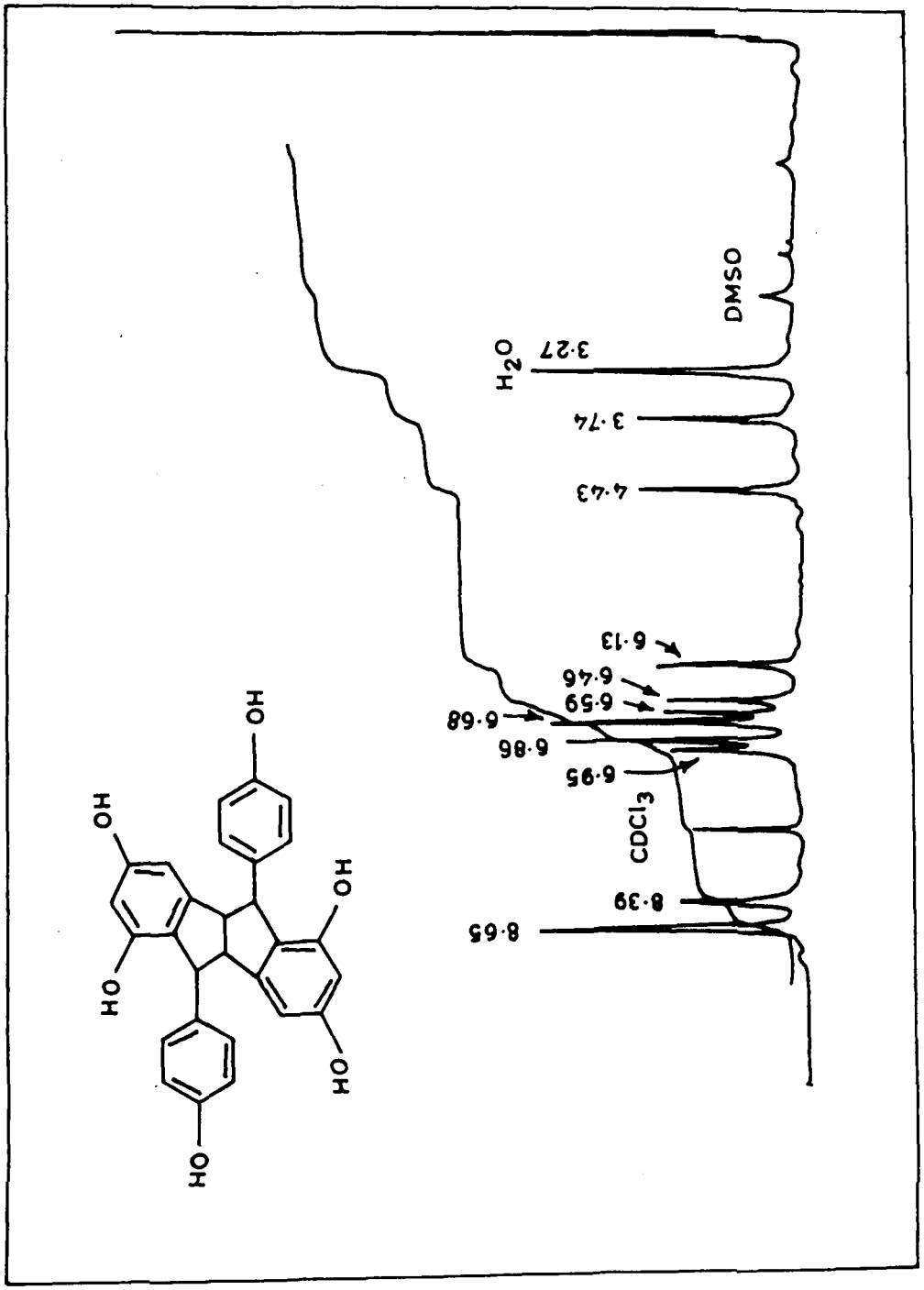
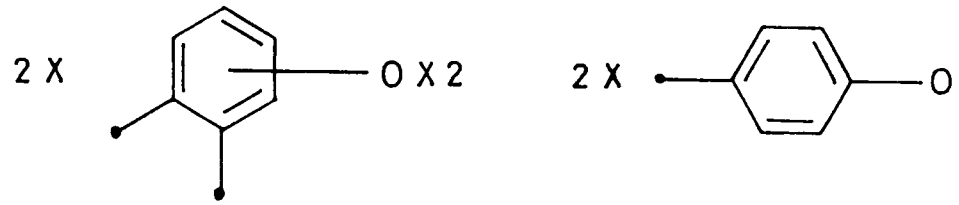

OH
OH
HO
OH
HO
HO
8·65
8·39
CDCl3
6·95
6·86
6·68
6·59
6·46
6·13
4·43
3·74
H2O 3·27
DMSO

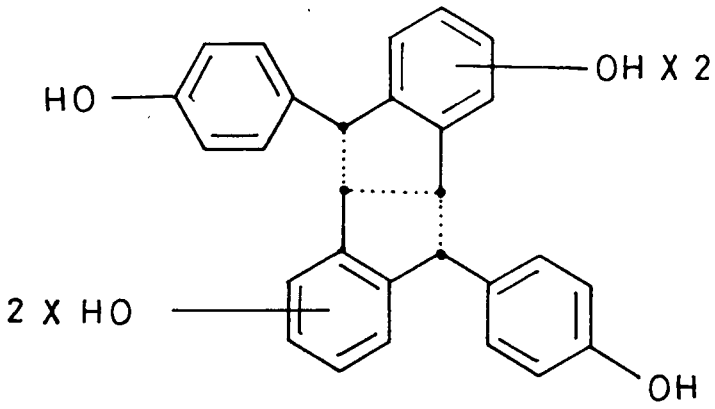
FIG. 22

contains 22 hydrogens of which 12 must be aromatic on the basis of the rise of integral over this region. The spectrum shows from low to high field two singlets integrating for 4 and 2 protons which disappear on $D_2O$ shake, as shown by an earlier spectrum, not reproduced here because of the presence of some impurities in the sample. The presence of six phenolic hydroxyl groups was later confirmed through preparation of derivatives. The OH resonances are followed by a pair of doublets (J=9 Hz) each integrating for 4 protons which indicates the presence of an AA'BB' system of protons in the molecule. There must, therefore, be two similar 1,4-disubstituted benzene rings in pallidol which explains the intense band at 835 $cm^{-1}$ in the IR spectrum. Next, there are two (2H) singlets with slight splitting as observed in the signals of meta coupled protons. There must, therefore, be 4 such protons so located as to form two sets. And, lastly between the signal of water, always present in traces in DMSO-$d_6$, and resonances of aromatic hydrogens there are two more signals each integrating for 2 protons. All the 22 hydrogens are thus satisfactorily accounted for in the NMR spectrum. The purity of the sample is evident from the neat integration as well as absence of signals of any impurities. It is obvious from this spectrum which contains two sets of equivalent ortho

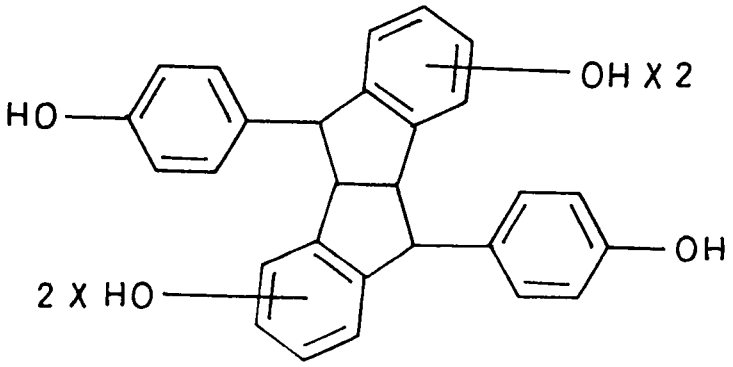
coupled protons and two sets of equivalent meta coupled protons that pallidol has a symmetrical structure. Further since there are six phenolic hydroxyls and 12 aromatic protons the spectrum of pallidol must incorporate atleast four aromatic rings. Since two of these rings are para substituted as indicated by the AA'BB' coupling pattern of 8 protons, each must have a hydroxyl group at position 1, position 4 being used for attachment to the rest of the molecule. This is substantiated also by the mass spectrum which shows strong loss of phenol from the molecular ion. The other two aromatic rings must share the four hydroxyl groups equally, because of the requirement of symmetry, and the hydroxyls must be meta to each other since the compound does not give a distinct ferric colour; the NMR spectrum shows, besides, that the 4 protons, two on each ring also bear meta relationship. On the basis of this data pallidol can be assumed to have the part structure (XLIV) which adds upto 24 aromatic and 6 benzylic carbons, that is two more than permitted by the molecular formula $C_{28}H_{22}O_6$. An adjustment of benzylic carbons to 4 is possible if it is assumed that two of these are shared i.e. are doubly benzylic. Keeping in mind the symmetry of the molecule part structure (XLIV) can then be extended to (XLV) which can be completed to the tetracyclic structure (XLVI).




( XLIV )

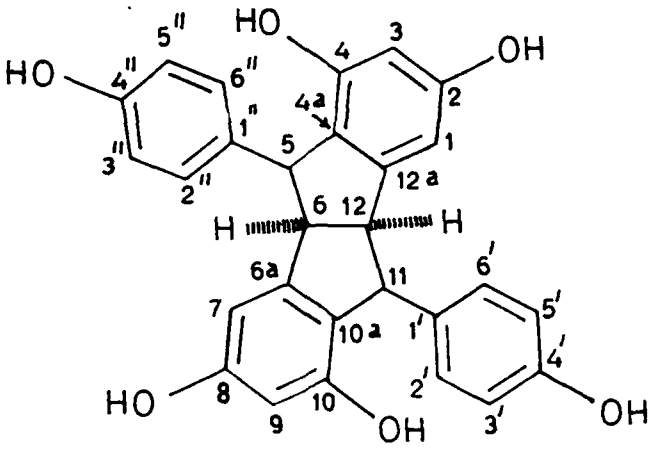



( XLV )

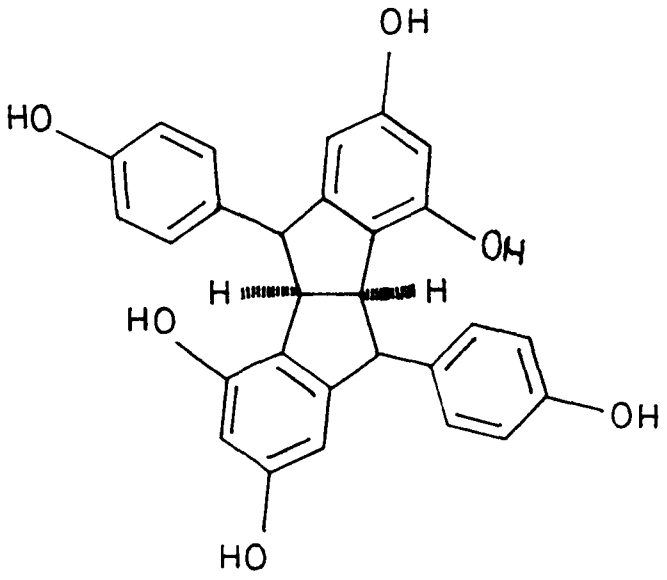



( XLVI )

The (2H) singlets at higher field are now reasonably assigned to the methine hydrogens which are equivalent in pairs. With regard to location of two hydroxyls in each tetrasubstituted ring there are two possibilities (XLVII) and (XLVIII). It is



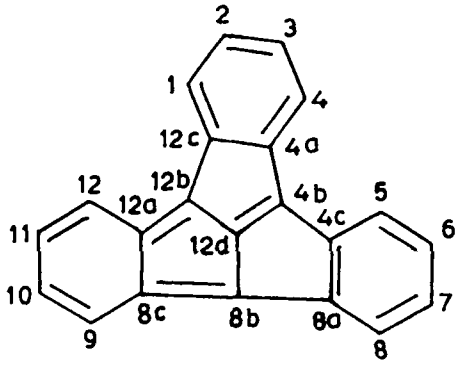

(XLVII)




(XLVIII)

impossible to distinguish between these two structures by spectroscopic means in the absence of any comparative data on similar compounds. Since both structures contain free positions *para* to two phenolic hydroxyl groups distinction on the basis of chemical reactivity is not feasible either; the amount of material in hand was, in any case, not sufficient for this approach. Both structures are obviously derived from condensation of two identically substituted stilbene units. In (XLVII), the combining units are of resveratrol (3,4',5-trihydroxy-*trans*-stilbene) which is of common occurrence and which alone, as shown later, can undergo oxidative coupling to give rise to a symmetrical dimer. (XLVIII) can, therefore, safely be excluded on biogenetic grounds. Taxonomic considerations also point the same way due to isolation of ε-viniferin, a resveratrol dimer from fungal infected *Vitis vinifera*.

Structure (XLVII) shows pallidol to be a dibenzopentalene derivative of a type so far unknown as a natural product. It is numbered, therefore, as an indan derivative, the analogy being that of dibenzo[2,3:4,5]pentaleno[1,6-ab] indene (XLIX)[59].




( XLIX )

Returning to the NMR spectrum the (4H) singlet at 8.65 can be assigned to hydroxyls at positions 2,4,8 and 10 and the (2H) singlet at 8.39 to those at 4' and 4''. Positions 3',5',3'',5'' contain one set of equivalent protons and 2',6',2'',6'' the other and these together account for the AA'BB' doublets of 8 protons. The meta coupled doublets are reasonably assigned to the hydrogens on C-1, C-3 and C-7, C-9. The hydrogens on C-5, C-11 and C-6, C-12 are equivalent and account for the two (2H) singlets at the higher field.

Pallidol can be methylated by methyl iodide over potassium carbonate to give a crystalline hexamethyl ether which being soluble in chloroform allows measurement of the NMR spectrum in $CDCl_3$. The 60 MHz NMR spectrum (Fig. 23)

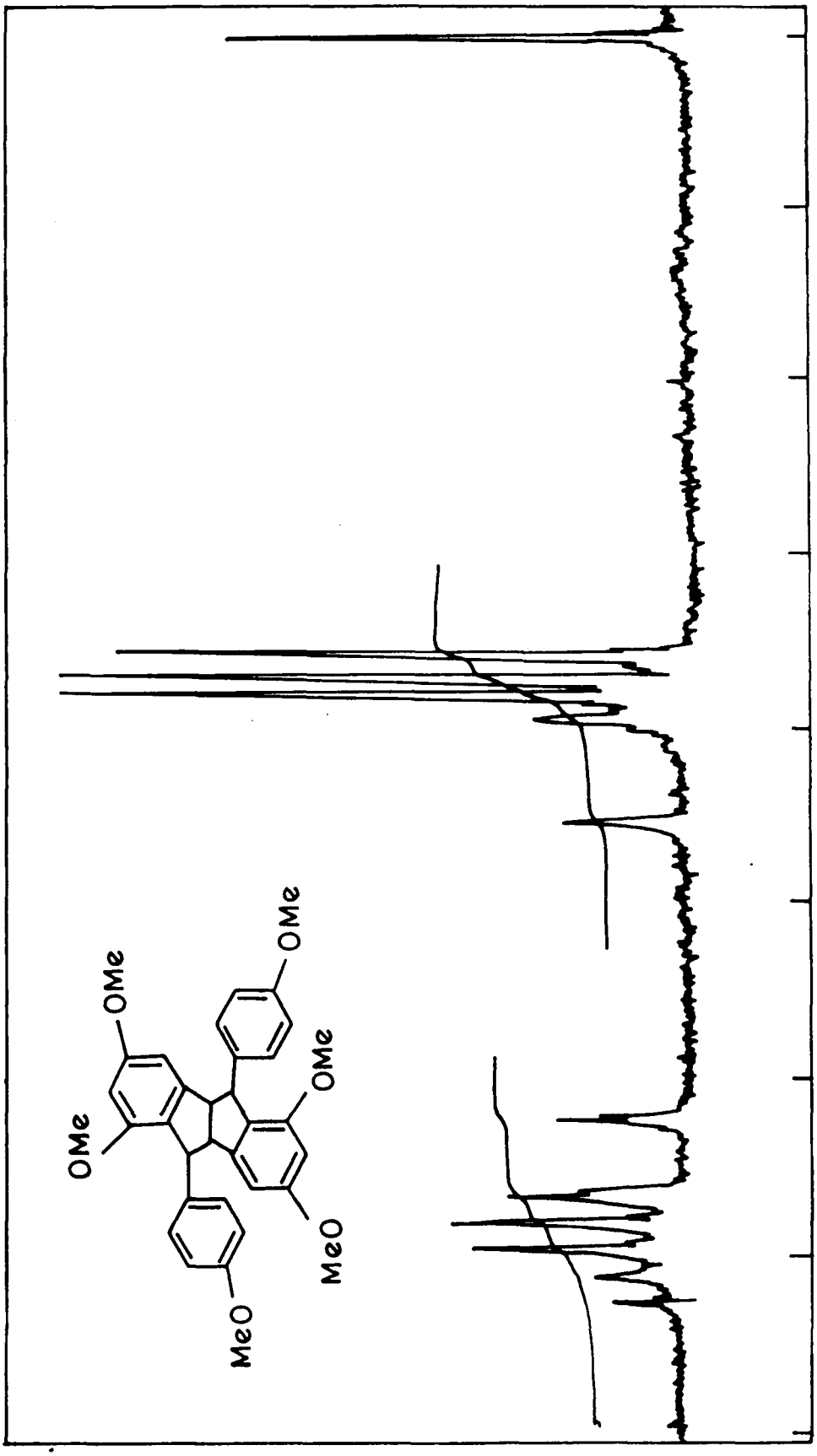



FIG. 23

shows the six methyls as three singlets at 3.55, 3.71 and 3.80 and the four methine hydrogens as two broad singlets. The aromatic region is not well resolved as in the spectrum of pallidol because of overlap of the signal of one set of meta coupled protons with part of the AA'BB' quartet. The points of interest in the spectrum are the shielding of two methoxyls and a slight shift to lower field of the two methine singlets, the possible reason for which will be discussed later.

The mass spectrum of pallidol (Fig. 24) shows that the molecular ion, and the fragments resulting from its rupture, have low abundance. This is understandable since pallidol has a high melting point (with decomposition) and consequently is not sufficiently volatile. Structurally the most important peak is at m/z 359 corresponding to elimination of phenol from the molecular ion. Another significant peak is at 227 which arises due to cleavage of the molecule into monomer stilbene units. In contrast to the pallidol spectrum, the mass spectrum of the methyl ether (Fig. 25) shows high abundance of the molecular ion and its major cleavage products. It can be rationalised as shown in scheme V.

Because of its molecular symmetry the $^{13}C$ NMR spectrum (Fig. 26) of pallidol shows only twelve carbon

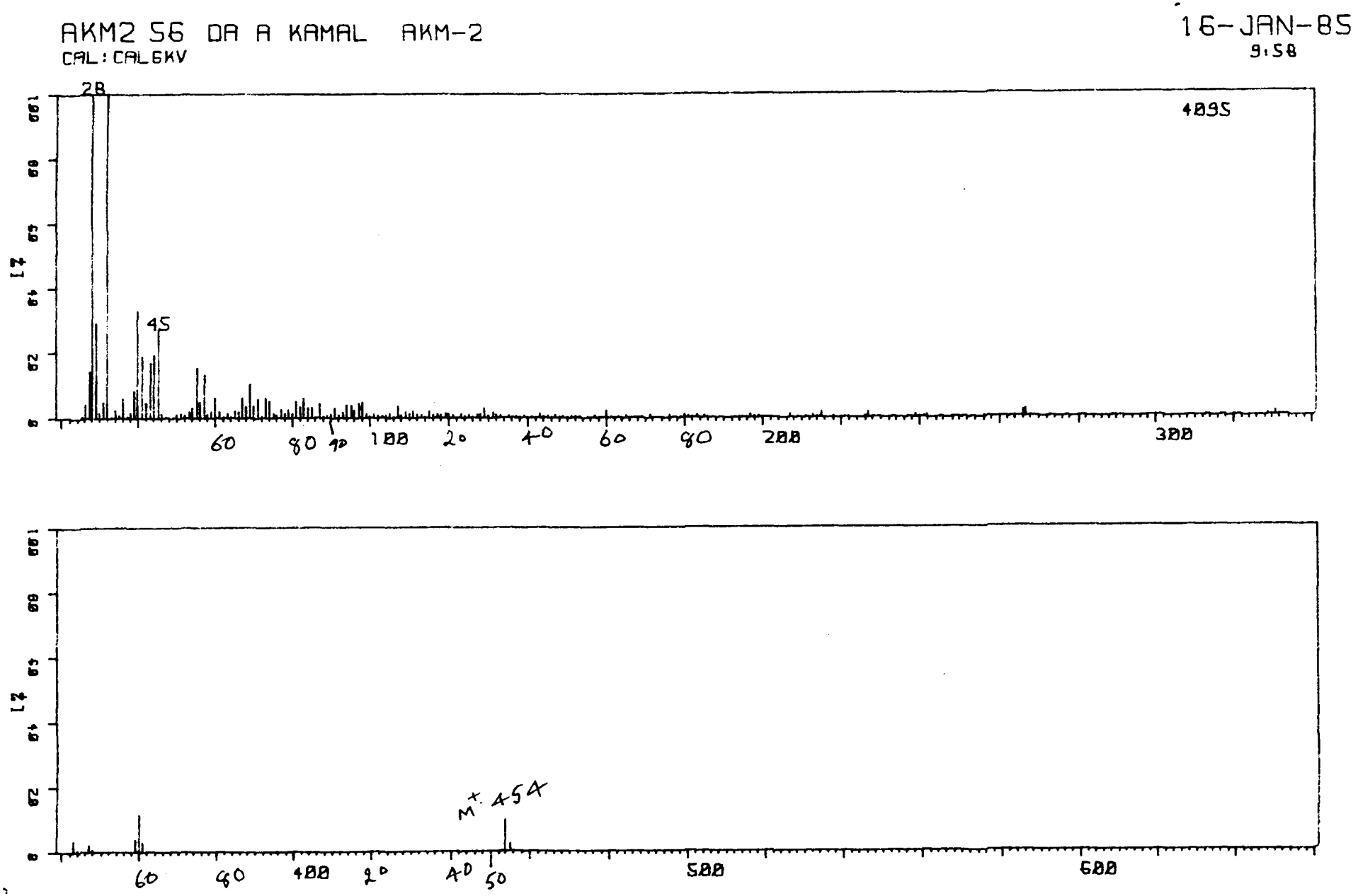

AKM2 56 DA A KAMAL AKM-2
CAL: CAL6KV
16-JAN-85
9:58
28
45
4095
%
60
80
100
120
140
160
180
200
300
M+ 454
500
600

FIG. 24

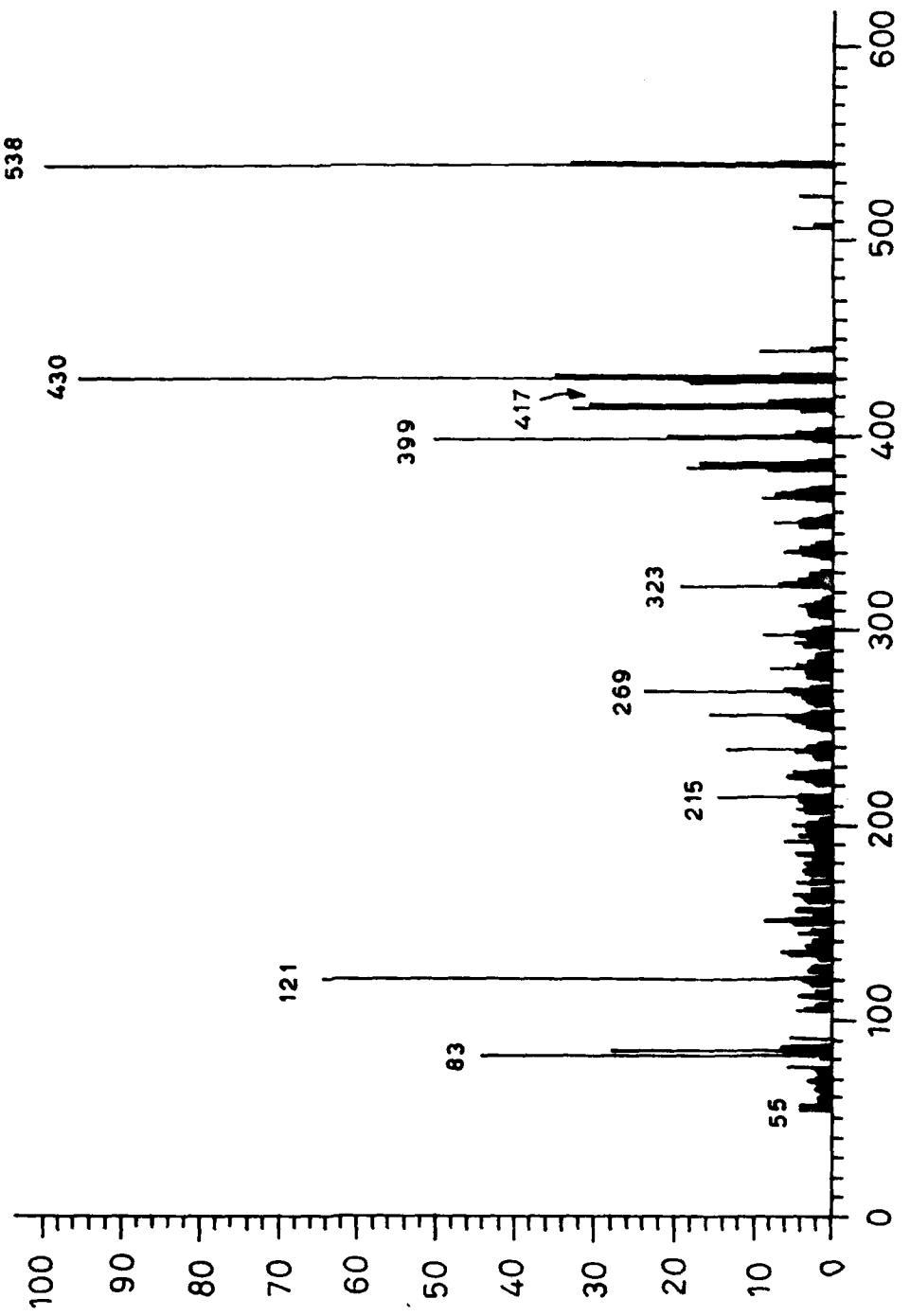
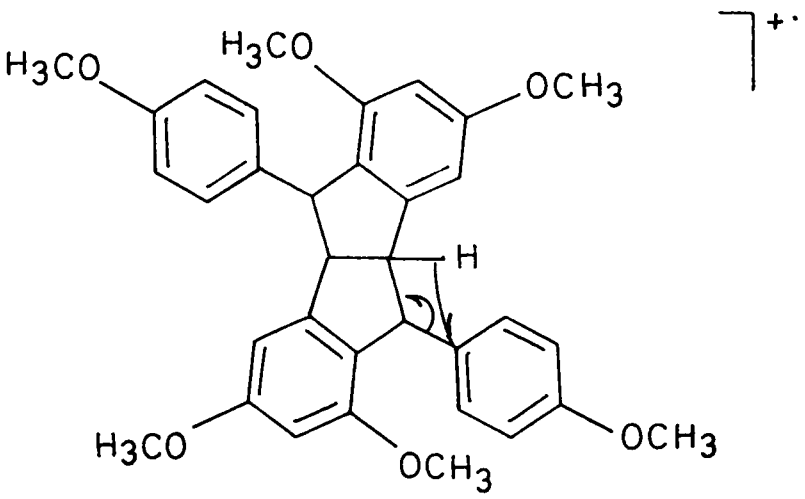
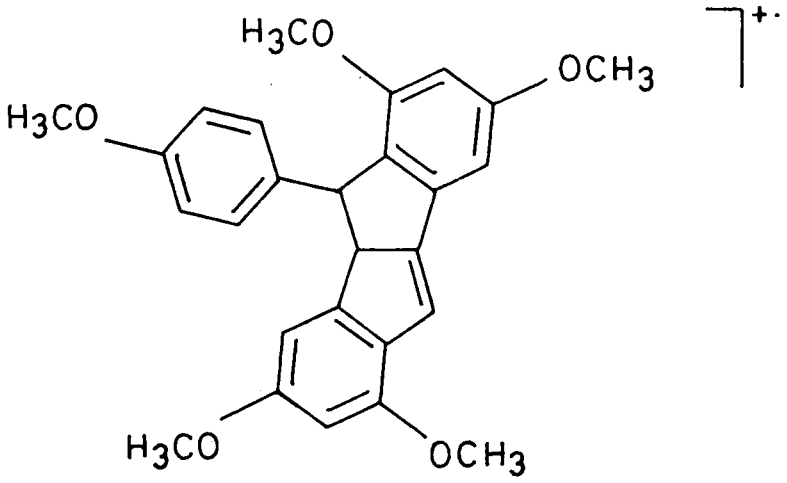

538
430
417
399
323
269
215
121
83
55
0
100
200
300
400
500
600
0
10
20
30
40
50
60
70
80
90
100

FIG. 25

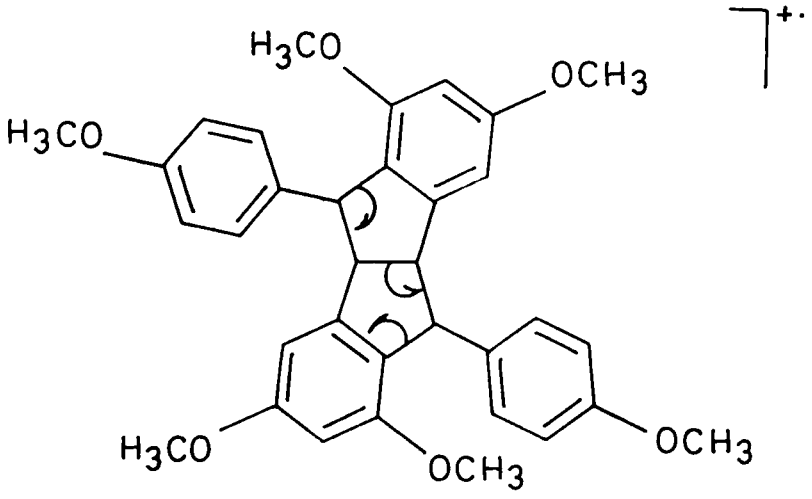



m/z 538 (100)

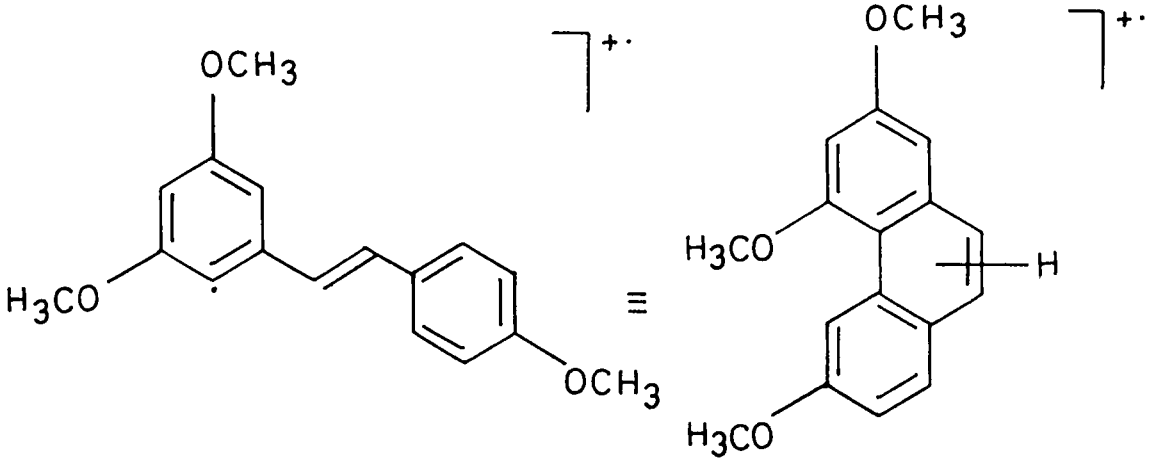



m/z 430 (95)

scheme V

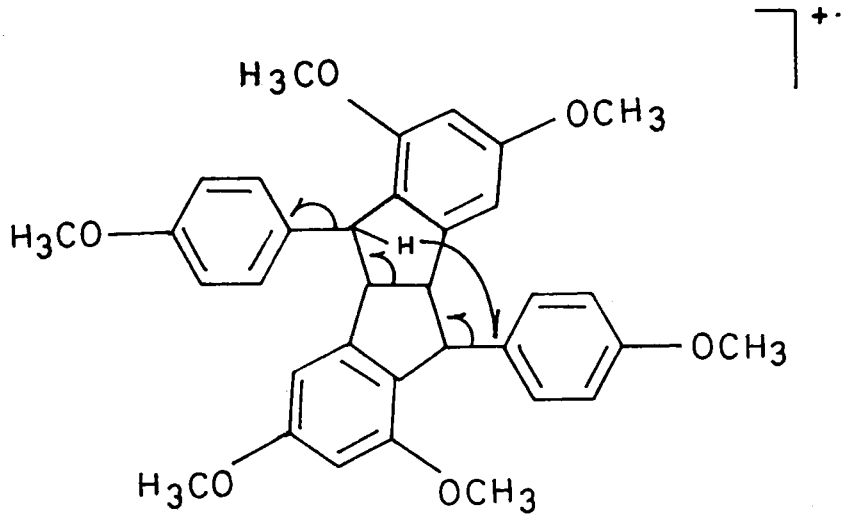



m/z 538 ( 100 )

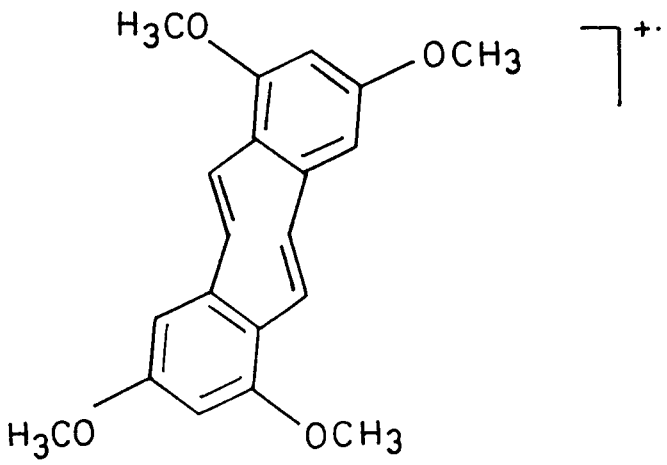



m/z 269 (29)

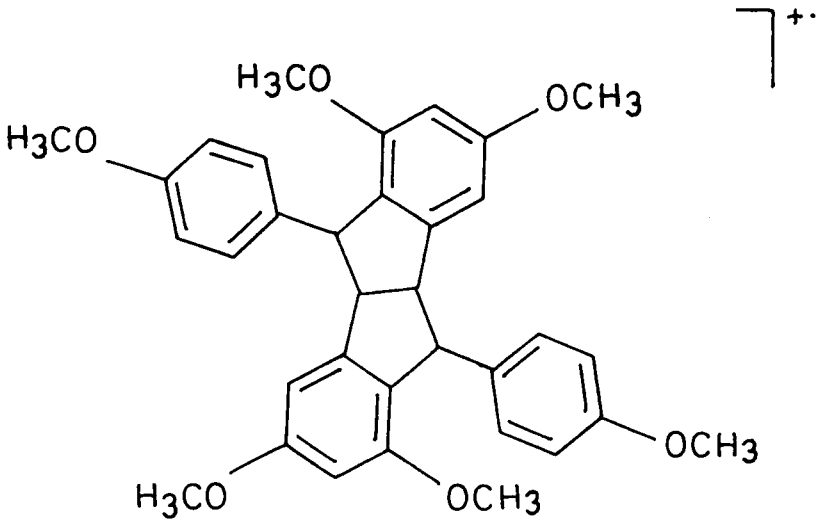
m/z 269



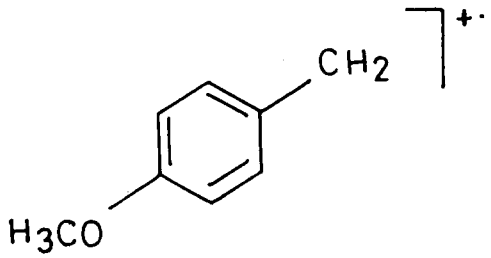

m/z 538 (100)



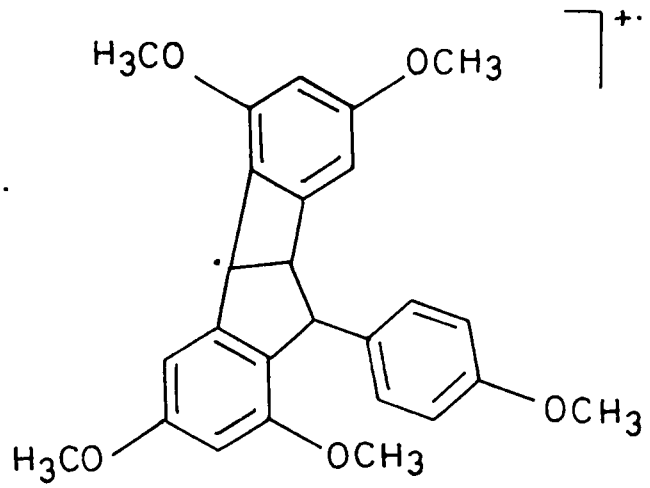

m/z 323 (24)




m/z 538 ( 100 )




m/z 121 ( 70 )




m/z 417 ( 55 )

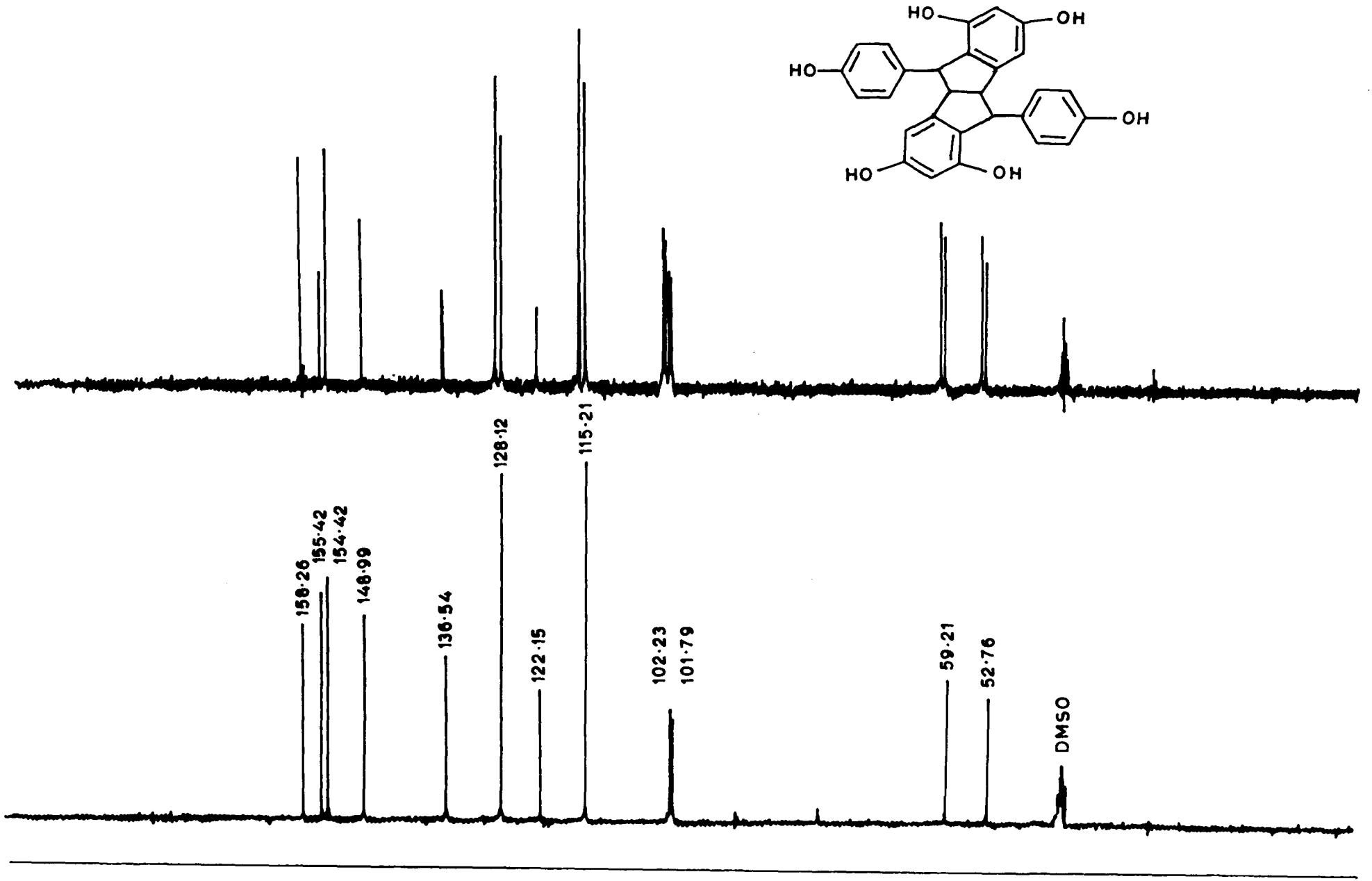
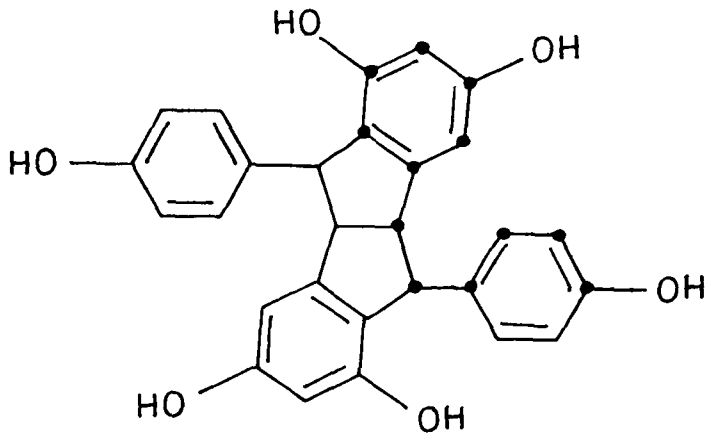

HO
OH
HO
OH
HO
OH
158·26
155·42
154·42
148·99
136·54
128·12
122·15
115·21
102·23
101·79
59·21
52·76
DMSO

FIG. 26

atoms. This number is less than half the total number of carbon atoms present in the molecule because of the added symmetry of the para substituted benzene rings which makes the ortho carbons on either side of the substituents also equivalent. The twelve carbons are indicated in (L); assignments shown in table 2 are made on the basis of data available in literature on phenol derivatives and specifically the assignments made in copalliferol-A (XXXVII)[50] which also contains one indan system. The assignments check with the results of SFORD but some values are, of course, interchangeable as pointed out in table 2. The gross structure of pallidol is thus firmly established and only its stereochemistry has to be settled.




(L)

Table 2

$^{13}C$ NMR chemical shifts of pallidol (XXXIV) and its acetate.

| Assignment | Pallidol [DMSO-$d_6$] | Pallidol acetate [$CDCl_3$] |
|---|---|---|
| C-1 | 102.2 (d) | 115.3 (d) |
| C-2 | 154.4 (s) | *147.9 (s) |
| C-3 | 101.7 (d) | 115.0 (d) |
| C-4 | 158.2 (s) | *151.1 (s) |
| C-4a | 122.1 (s) | 133.5 (s) |
| C-5 | 52.7 (d) | 55.8 (d) |
| C-6 | 59.2 (d) | 61.1 (d) |
| C-6a | 148.9 (s) | 147.5 (s) |
| C-7 | 102.2 (d) | 115.3 (d) |
| C-8 | 154.4 (s) | *147.9 (s) |
| C-9 | 101.7 (d) | 115.0 (d) |
| C-10 | 158.2 (s) | *151.1 (s) |
| C-10a | 122.1 (s) | 133.5 (s) |
| C-11 | 52.7 (d) | 55.8 (d) |
| C-12 | 59.2 (d) | 61.1 (d) |
| C-12a | 148.9 (s) | 147.5 (s) |
| C-1' | 136.5 (s) | 140.7 (s) |
| C-2' | 128.1 (d) | 128.6 (d) |

| | | |
|---|---|---|
| C-3' | 115.2 (d) | 121.9 (d) |
| C-4' | 155.4 (s) | *149.6 (s) |
| C-5' | 115.2 (d) | 121.9 (d) |
| C-6' | 128.1 (d) | 128.6 (d) |
| C-1'' | 136.5 (s) | 140.7 (s) |
| C-2'' | 128.1 (d) | 128.6 (d) |
| C-3'' | 115.2 (d) | 121.9 (d) |
| C-4'' | 155.4 (s) | *149.6 (s) |
| C-5'' | 115.2 (d) | 121.9 (d) |
| C-6'' | 128.1 (d) | 128.6 (d) |
| $\underline{C}H_3CO$ | | 19.9 (q) |
| | | 20.9 (q) |
| | | 21.0 (q) |
| Me$\underline{C}$O | | 167.6 (s) |
| | | 168.8 (s) |
| | | 169.2 (s) |

---

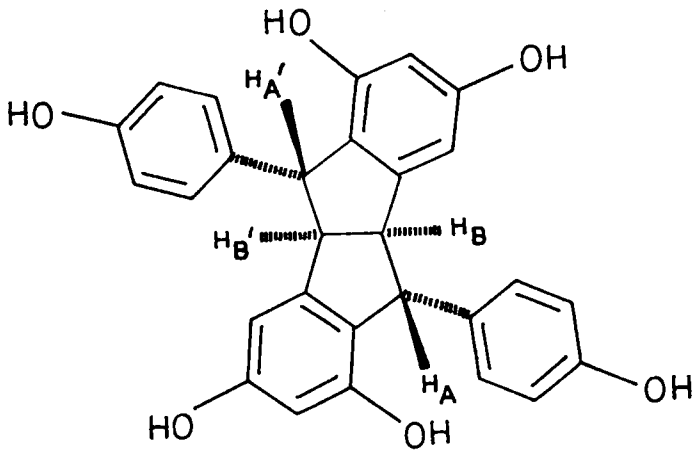
In $\delta$ values from TMS (100 MHz, in DMSO-$d_6$ and $CDCl_3$).

SFORD multiplicities in parentheses.

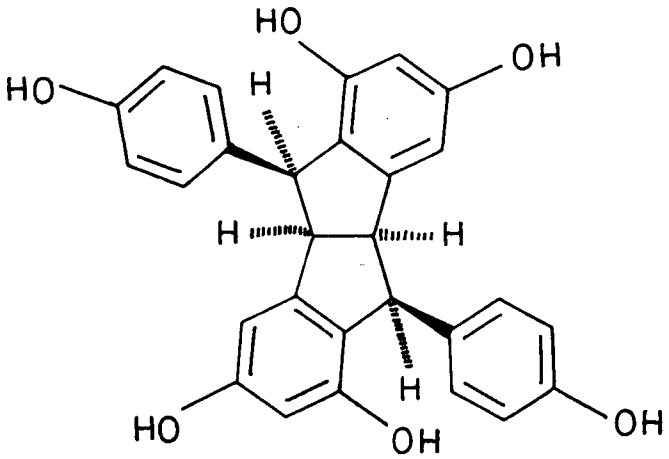
*Values may be interchanged but those given here are considered to be most likely.

Stereochemistry

Among natural products containing fused 5-membered ring systems such as lignans[60-68] only the cis form is encountered because of the high degree of strain in the trans fused systems. If the requirement of symmetry is taken into account only two structures (XXXIV) and (LI) are possible for pallidol. In (XXXIV) the para-hydroxyphenyl groups are equatorial and consequently the adjacent methine hydrogens are trans. In (LI) these groups are axial which makes all the methine hydrogens cis. In the absence of comparative data on similar systems it is not possible to decide conclusively between these two alternatives on the basis of chemical shifts of aromatic protons or methine hydrogens alone. The NMR spectra of the methyl ether and acetate are stereochemically more informative and are characterised by the high field position of two methyl singlets. Both spectra, compared to the spectrum of pallidol, show convergence of the methine singlets which is more pronounced in the acetate spectrum. Another special feature of the acetate spectrum is the clear splitting of the two singlets into double doublets. Whereas change of solvent from $CDCl_3$+DMSO-$d_6$ for pallidol to neat $CDCl_3$ for methyl ether and acetate may have had some influence on chemical shifts, the larger shift in the case of the

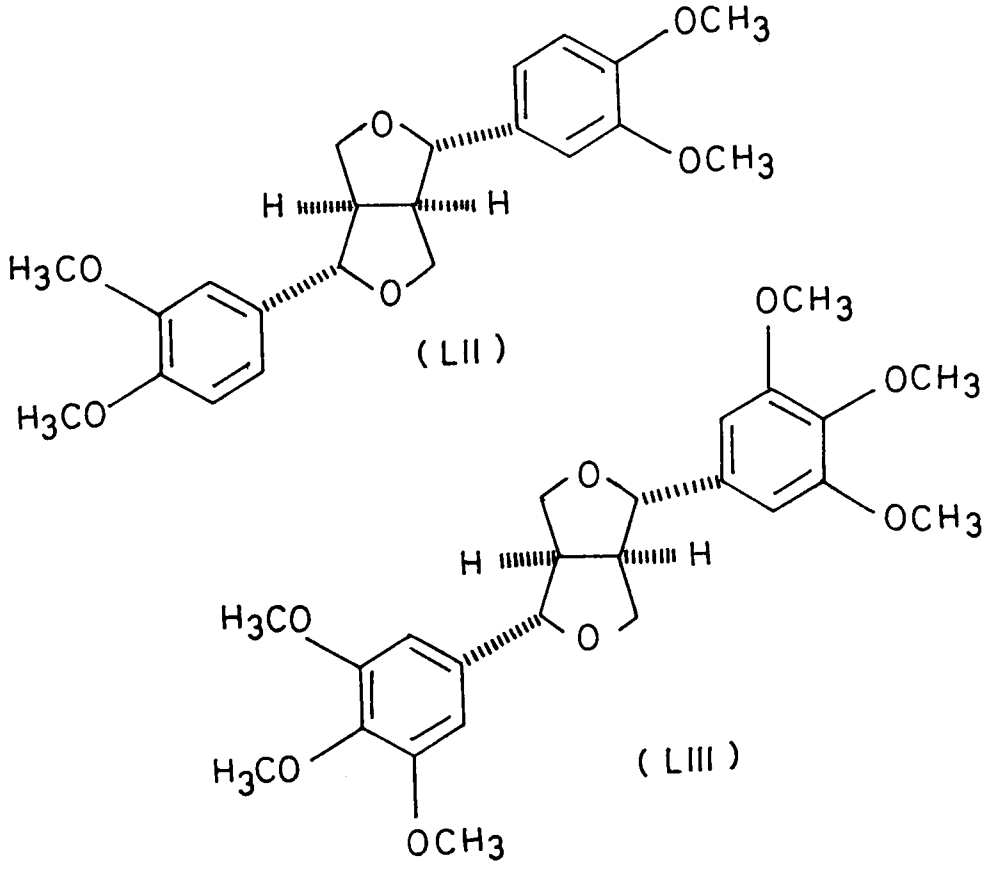



( XXXIV )




( LI )

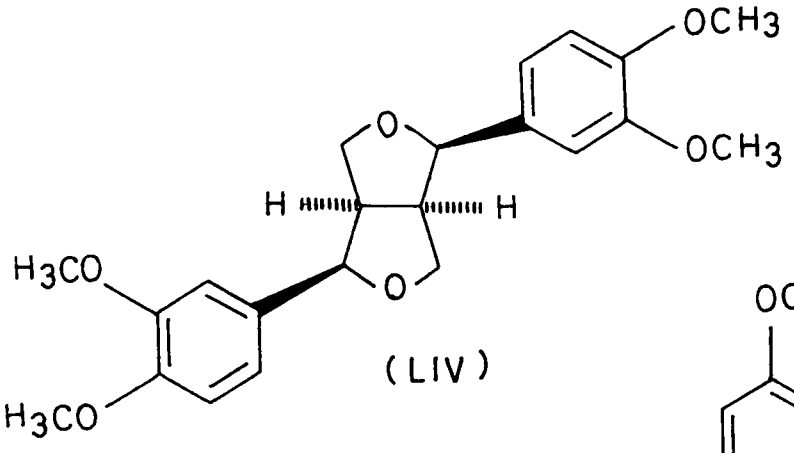
acetate can have only structural significance. Dreiding models of pallidol show that the axial aryl groups experience considerable hinderance to free rotation and should be rigidly held in a more or less perpendicular orientation of the plane of the aryl rings and the indan system. The situation is similar to that in lignans having dioxabicyclo[3.3.0]octane ring system, the NMR spectra of which have received much attention from Pelter and co-workers[69-74]. They found that when Stuart models of these lignans were made the crowding was so great that the axial methylene hydrogens could not be easily introduced. They further noted that the chemical shift of these hydrogens were different in diequatorial (LII, LIII) and diaxial (LIV, LV) series[75,76], and assigned the diaxial structure to compounds in which the concerned hydrogens appear at higher field. They observe that whereas they found less crowding in the models of the diequatorial isomer the aryl rings were still not free to rotate and caused deshielding of both methylene hydrogens to different degrees. This means that the aryl groups of the diequatorial isomer must interact peripherally i.e. side ways with the equatorial methylene hydrogens and equatorial benzene rings must, therefore, be roughly coplanar with the fused ring system. The steric situation is similar in pallidol the crowding being further



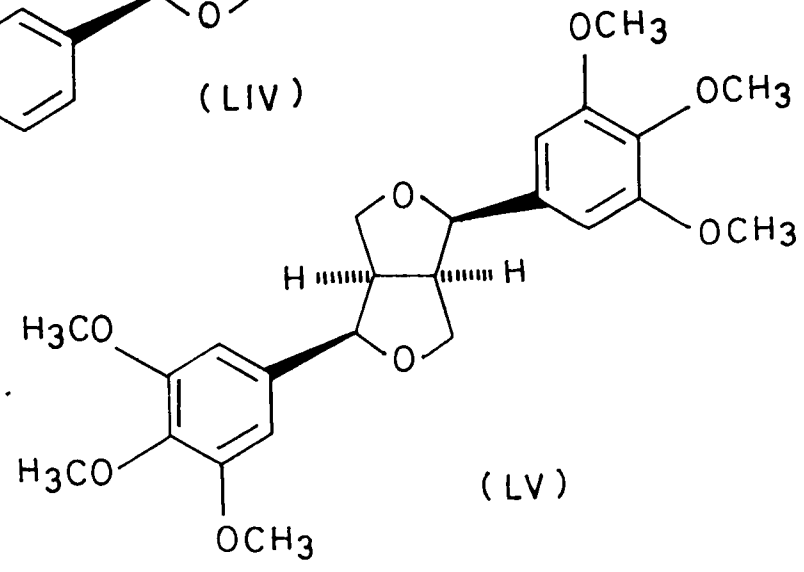
( LII )



( LIII )



( LIV )



( LV )

accentuated by fusion of two more benzene rings which results in a cage like molecular framework. There are, however, no methylene hydrogens, the concerned carbons forming part of the indan nucleus on either side, but C-4 and C-10 occupy roughly the same region as the methylene hydrogens in lignans and if they carry methoxyl and acetyl substituents the chemical shifts of the methyls should be influenced by the conformation of the aryl groups on C-5 and C-11. Models show that if these groups are axial they must have the same conformation as in lignans and should shield the methoxyl and acetoxyl groups on C-4 and C-10. On the other hand, again by analogy with lignans, one would expect a slight deshielding of the substituents when the aryl groups are diequatorial. Models, however, show that the parallel with lignans does not hold in this case and the orientation of the aryl groups which would have a deshielding influence on the substituents on C-4 and C-10 becomes even more untenable if these carbons carry large substituents because of severe interaction between these and the ortho-aryl hydrogens. The favoured orientation is thus the same as in the axial case with the planes of the two ring systems bisecting each other. This not only puts the opposite methyls of the acetate or the methoxyl functions in the shielding zone of the benzene ring it also places the methine

hydrogens in the peripheral region of the aryl groups. Since there is not only shielding of the two methyls but also change in the chemical shift of the two methine hydrogens the aryl groups are reasonably assumed to be equatorial.

Of the two singlets the one at higher field should reasonably be assigned to the bridge-head hydrogens since they are deshielded by only one benzene ring. There is, however, a possibility that the doubly benzylic i.e. C-5, C-11 protons are shifted upfield due to shielding by the fused benzene rings which is feasible in view of the slight curvature of the molecule. Whatever the assignments the hydrogens responsible for the high field signal are deshielded in the change from phenol to methyl ether to acetate. This means that introduction of bulky substituents on C-4 and C-10 enhances the conformational rigidity of the aryl groups and they are so displaced as to deshield either the doubly benzylic or bridge-head hydrogens. It is obvious that this effect is possible only in the case of the diequatorial disposition of the aryl groups and the relative stereochemistry as shown in (XXXIV) is further confirmed.

Polarimetric measurements showed pallidol to be optically inactive which came as a surprise in view of the

$C_2$ symmetry of the molecule. Racemic lignans[77,78] have been reported in literature but of the few oligomeric stilbenes all are optically active.

A further point of interest in the acetate spectrum is the distinct splitting of the methine singlets as already referred to. This splitting is visible in the 90 MHz NMR spectrum (Fig. 27) but the triplets at 4.15 and 4.45 are not sufficiently well resolved to allow measurement of the coupling constants. In the 300 MHz NMR spectrum (Fig. 28) double doublets replace the triplets and the resolution enhanced spectrum permits measurement of coupling constants which are found to be 3.7 and 2.6 Hz. These double doublets have their origin in the vicinal and long range coupling of $H_A$ to $H_B$ and $H_{B'}$. Though it has been emphasised by various authors[79-82] that the Karplus equation is not reliable in the case of the 5-membered ring systems the coupling constants, nevertheless, approximate to this relationship as these are found to be larger for cis than for the trans protons. This criterion has, in any case, been adopted by Camps[83] in assigning the trans configuration to the compound he synthesised (LVI). In the case of this compound also the bridge-head and allylic protons appear as triplets (J=2.7 Hz) in the 100 MHz NMR spectrum[83,84]. The triplets have been rationalised by him by

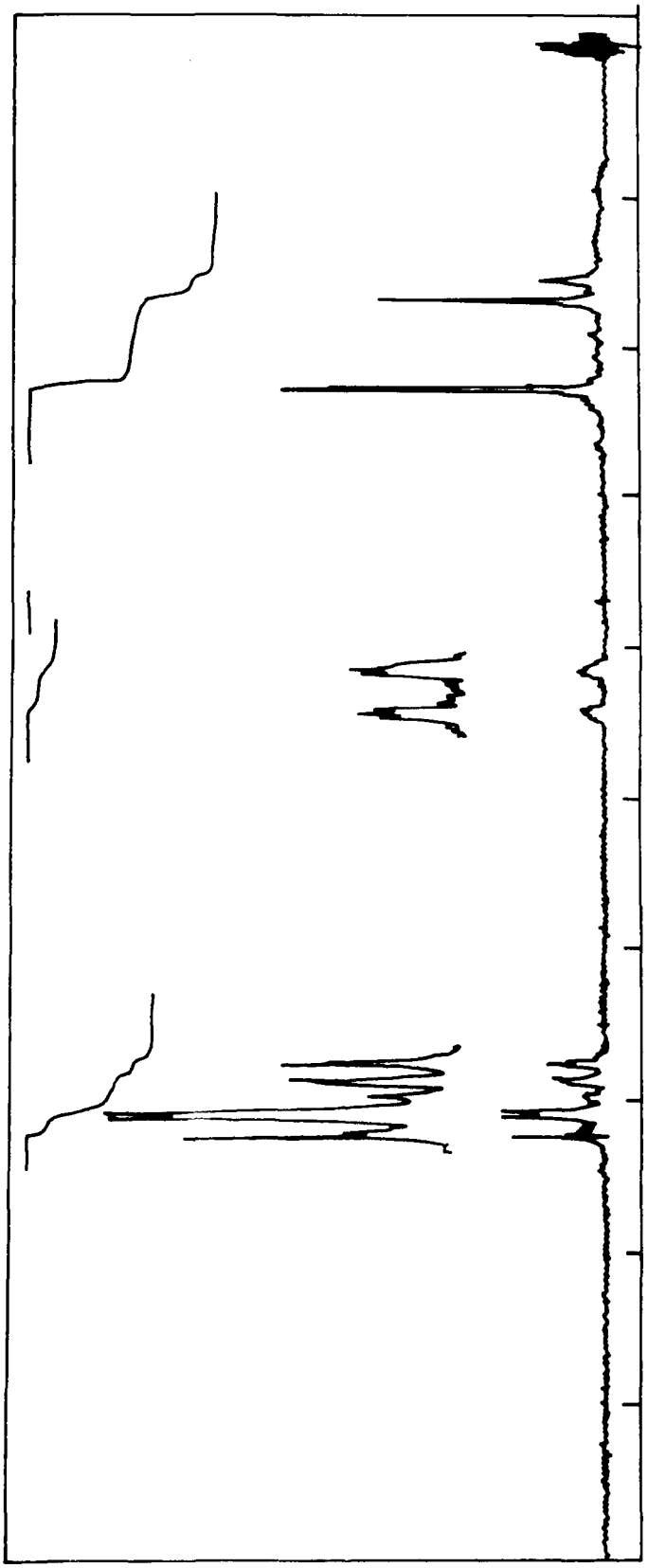



FIG. 27

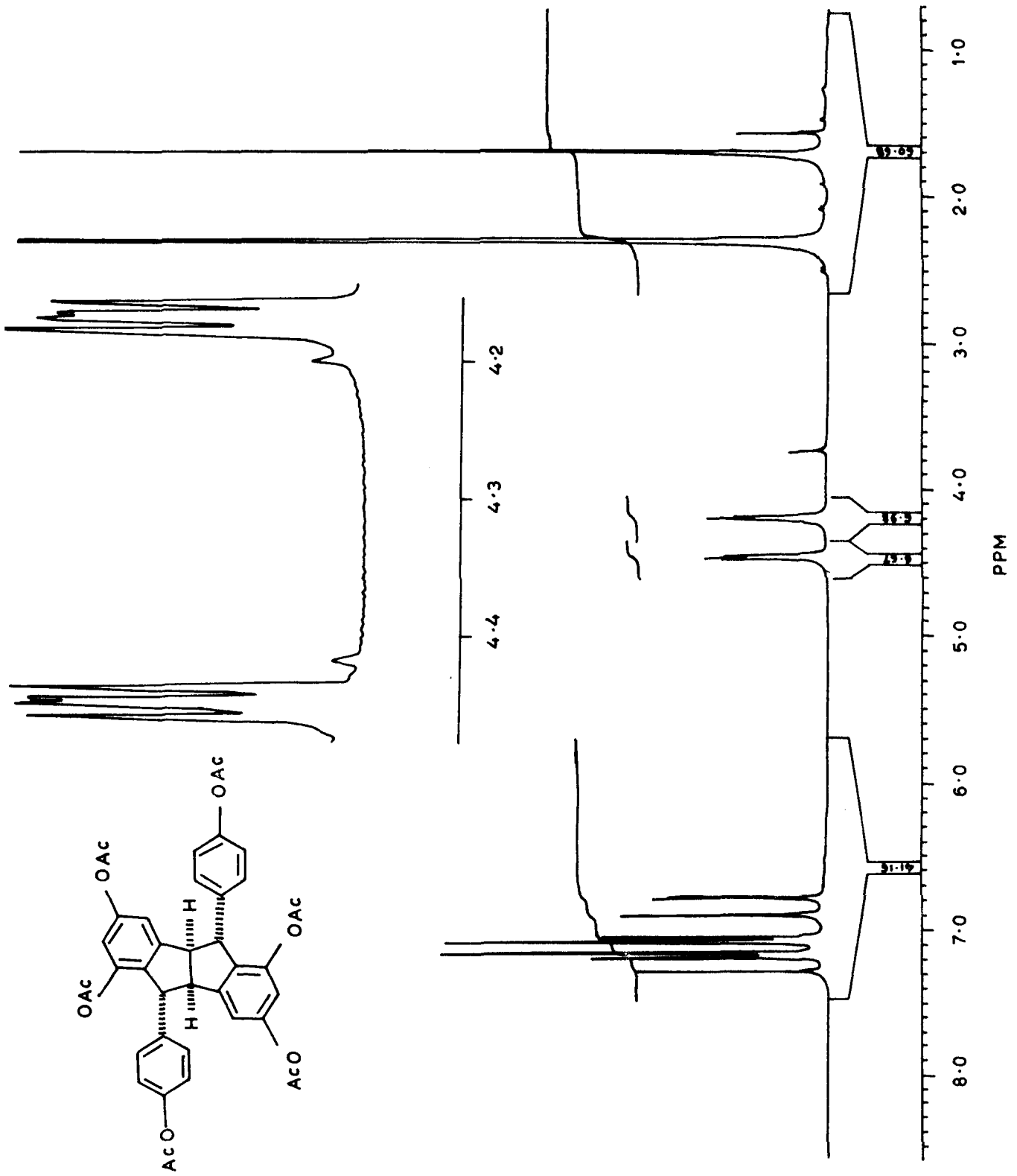
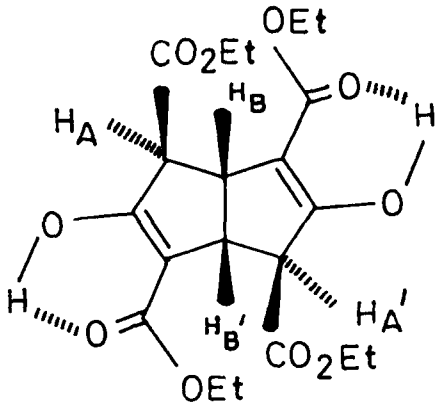



FIG. 28




(LVI)

postulating $J_{AB} \sim J_{AB'}$. Since this is approximately the apparent coupling constant in the 90 MHz NMR spectrum of pallidol hexaacetate it appears more or less certain that in his case also measurement at 300 MHz would have shown $J_{AB}$ to be unequal to $J_{AB'}$. The larger value, 3.7 Hz, must be assigned to the vicinal coupling. The long range coupling in (LVI) involves a homoallylic system[85] but in pallidol it is homobenzylic for which a J value of 2.6 Hz is unusual[86]. It is possible that the magnitude of the coupling is enhanced by the contribution of the $\sigma$-bonds i.e. it occurs also across the 5-membered ring system.

Measurement of $^{13}C$ NMR spectrum and results of SFORD (Fig. 29) of the acetate further establish that no molecular rearrangement has occurred during acetylation of pallidol as all the twelve carbon atoms appear at the expected values which are shown in table 2. It is difficult to be certain of the exact reason for the transformation of the methine singlets of pallidol to double doublets in the acetate but since there is no indication of splitting in the methyl ether spectrum molecular distortion is unlikely and perturbation due to proximity of carbonyl groups is probable.

The most likely mechanism for the biogenesis of pallidol, by analogy with that suggested for copalliferol-A[50], is oxidative coupling of two resveratrol units as shown in scheme VI.

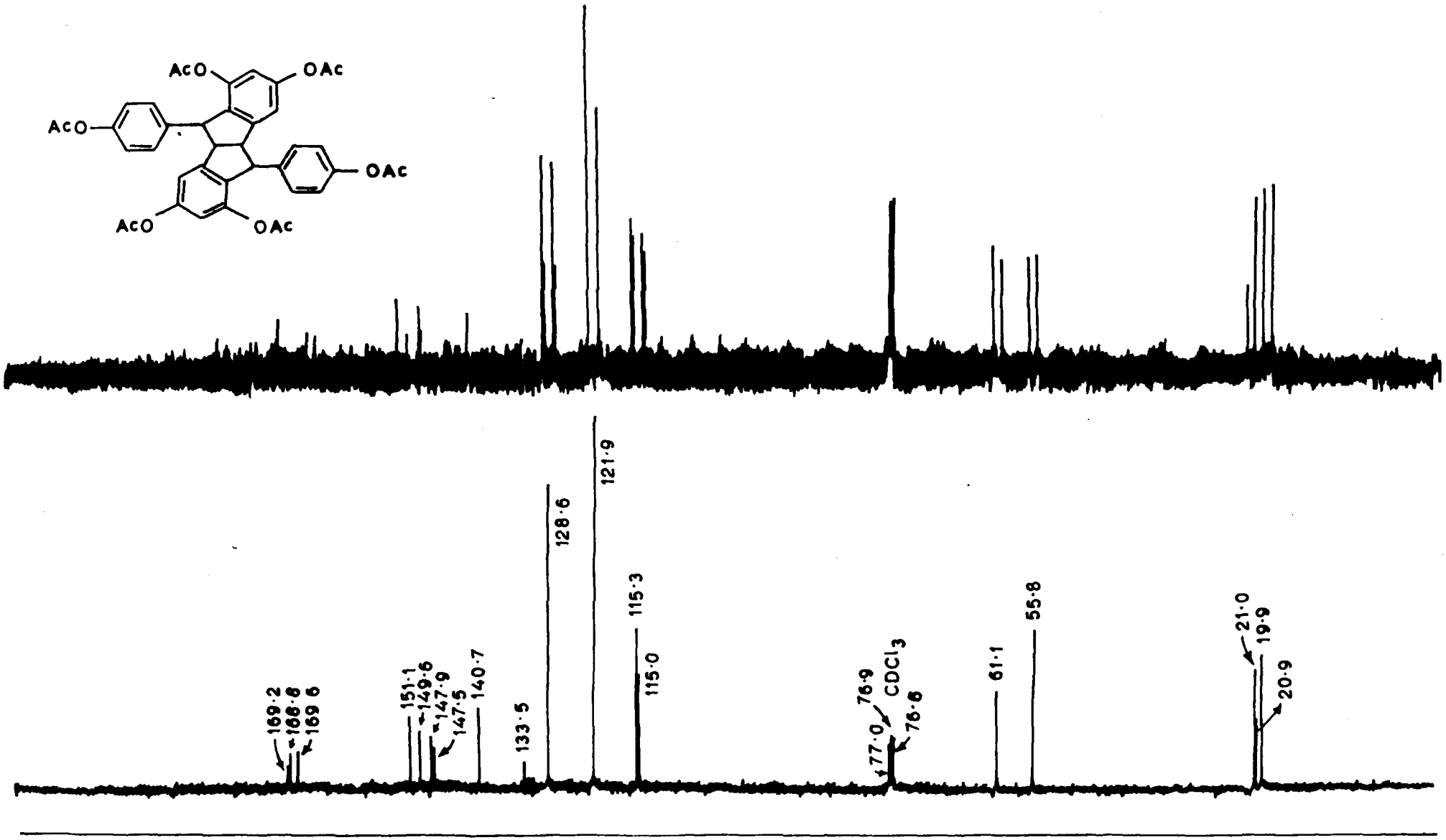
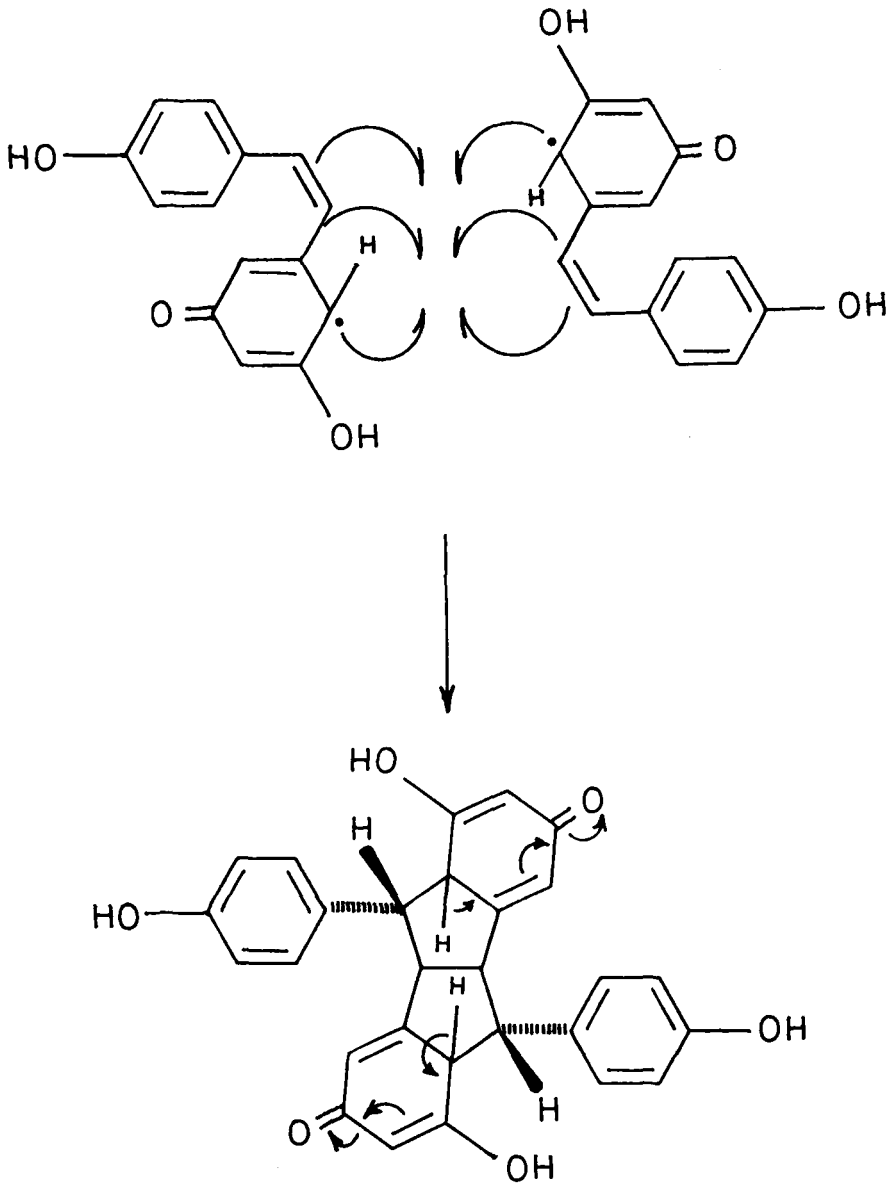

AcO
OAc
AcO
OAc
AcO
OAc
169·2
168·8
169·6
151·1
149·6
147·9
147·5
140·7
133·5
128·6
121·9
115·3
115·0
76·9
77·0
CDCl3
76·6
61·1
55·8
21·0
19·9
20·9

FIG. 29




( XXXIV )

scheme VI

# E X P E R I M E N T A L

## EXPERIMENTAL

The melting points were taken on a Kofler block and Reichert Thermovar and are uncorrected. The UV spectra were recorded in 95% methanol using Unicam PU 8800 spectrometer. IR spectra were measured on Unicam SP3-100 and Perkin-Elmer 275 spectrometers. $^{1}H$ NMR, $^{13}C$ NMR and mass spectra were recorded at Central Drug Research Institute, Lucknow and Regional Research Laboratories, Hyderabad on different instruments and the field strength is indicated. The 270 MHz NMR spectra were provided by Indian Institute of Science, Bangalore. The high resolution mass and 300 MHz NMR spectra were recorded at H.E.J. Research Institute of Chemistry, Karachi through the courtesy of Prof. Atta-ur-Rahman. The chemical shifts are reported in $\delta$ values relative to TMS assigned at zero.

BDH and E. Merck Analar solvents were used for chromatographic purposes without prior distillation. Solvents used for extraction were purified by standard procedures. Column chromatography was carried over BDH silica gel (60-120 mesh) and TLC over silica gel GF 254 (E. Merck).

## Gnetum ula

### Extraction and Isolation

Stem-wood (5 kg) of the plant Gnetum ula was chopped into small pieces and extracted with petroleum ether at room temperature. The extract was discarded as it contained only oils and fats. The defatted plant material was then extracted with acetone in a Soxhlet for one week. The black resinous residue obtained after removal of the solvent under reduced pressure was taken up in water and exhaustively extracted with ethyl acetate in a liquid-liquid extractor. Evaporation of the solvent left a sticky brown residue (25 g) which was subjected to column chromatography over silica gel. Elution with petroleum ether afforded only oily material whereas elution with pure benzene gave a viscous mass which was found to be a mixture of several components and worked up separately.

### Methoxy stilbene (VI)

Elution of the column with benzene-ethyl acetate (80:20) afforded a light brown solid which was repeatedly crystallised from acetone-petroleum ether as a colourless

crystalline compound (300 mg), m.p.167-168°, $C_{15}H_{14}O_4$ ($M^{+\cdot}$ m/z 258).

Spectral data

UV(MeOH) $\lambda_{max}$ : 220, 330 nm.

IR(Nujol) $\nu_{max}$ : 3300 (br), 1595, 1420, 1350, 1030 $cm^{-1}$.

$^1H$ NMR(90 MHz) [DMSO-$d_6$] : $\delta$ 3.85 (3H, s, OMe), 6.16 (1H, br s, ArH-2'), 6.42 (2H, br s, -CH=CH-), 6.78 (1H, d, J=8 Hz, ArH-5), 6.9-7.20 (3H, m, ArH-4',5',6'), 7.40 (1H, d, J=8 Hz, ArH-6), 9.2 (3xOH, exchangeable on addition of $D_2O$).

$^1H$ NMR(100 MHz) [DMSO-$d_6$] : $\delta$ 3.85 (3H, s, OMe), 6.15 (1H, br s, ArH-4'), 6.43 (2H, d, J=2 Hz, ArH-2',6'), 6.76 (1H, d, J=8 Hz, ArH-3), 6.9-7.06 (3H, m, -CH=CH-; ArH-2), 7.19 (1H, d, J=2 Hz, ArH-6), 9.20 (3H, br s, 3xOH).

$^1H$ NMR(270 MHz) [DMSO-$d_6$] : $\delta$ 3.82 (3H, s, OMe), 6.12 (1H, br s, ArH-4'), 6.40 (2H, d, J=2 Hz, ArH-2',6'), 6.75 (1H, d, J=8 Hz, ArH-3), 6.90, 7.03 (2H, dd, J=17 Hz, -CH=CH-), 6.96 (1H, dd,

J=8,2 Hz, ArH-2), 7.16 (1H, d, J=2 Hz, ArH-6), 9.30 (3H, br s, exchangeable on addition of $D_2O$, 3xOH).

$^1H$ NMR(270 MHz) [DMSO-$d_6$+benzene-$d_6$] : $\delta$ 3.83 (3H, s, OMe), 6.28 (1H, dd, J=2 Hz, ArH-4'), 6.53 (2H, d, J=2 Hz, ArH-2',6'), 6.83 (1H, d, J=8 Hz, ArH-3), 6.94, 7.03 (2H, dd, J=17 Hz, -CH=CH-), 6.99 (1H, dd, J=8,2 Hz, ArH-2), 7.20 (1H, d, J=2 Hz, ArH-6), 9.35 (3H, br s, exchangeable on addition of $D_2O$).

Hydrogenation of methoxy stilbene

A solution of methoxy stilbene (100 mg) in methanol (25 ml) was hydrogenated over Pd/C. The viscous product was found to be a mixture and chromatographed over silica gel to give the dihydro derivative of the methoxy stilbene as an oil, $C_{15}H_{16}O_4$ ($M^{+\cdot}$ m/z 260), 260 (6), 232 (60), 153 (5), 138 (95), 123 (50), 107 (100).

Synthesis of 3,3',4,5'-tetramethoxy-trans-stilbene (IX)

3,5-Dimethoxyphenyl acetic acid (1.96 g) and 3,4-dimethoxybenzaldehyde (1.66 g) were condensed in presence

of piperidine (0.25 ml) at 160-175° for 16 hours. The reaction mixture was cooled and taken in methylenechloride and filtered. The methylenechloride soluble fraction was then extracted with dil. HCl until all the piperidine was removed. The organic layer was exhaustively extracted with 5% aqueous sodium hydroxide and the methylenechloride layer was washed several times with water and evaporated to yield a gum (1.0 g) which was subjected to column chromatography to give 3,3',4,5'-tetramethoxy-trans-stilbene as colourless oil; $C_{18}H_{20}O_4$ ($M^{+\cdot}$ m/z 300).

Spectral data

$^1$H NMR(60 MHz) [$CDCl_3$] : $\delta$ 3.80 (9H, s, 3xOMe), 3.85 (3H, s, OMe), 6.0-7.0 (8H, m, ArH; -CH=CH-).

3,3',4,5'-Tetramethoxy-trans-stilbene was found identical with the permethyl ether of the methoxy stilbene. The combined sodium hydroxide extract was neutralised with dil. HCl and the precipitate obtained was filtered, dried and crystallised from methanol to give colourless needles of the stilbene-α-carboxylic acid (600 mg), m.p.180-182°; $C_{19}H_{20}O_6$ ($M^{+\cdot}$ m/z 344).

Spectral data

$^1$H NMR(60 MHz) [$CDCl_3$] : $\delta$ 4.10 (6H, s, 2xOMe), 4.15 (6H, s, 2xOMe), 6.0-7.5 (7H, m, ArH; -CH=), 10.80 (1H, br s, exchangeable on addition of $D_2O$).

Decarboxylation of 3,3',4,5'-tetramethoxy-trans-stilbene-α-carboxylic acid (X)

The stilbene-α-carboxylic acid (300 mg) was refluxed with $CuCO_3$ (150 mg) in quinoline (10 ml) for about 2 hours. The reaction mixture was cooled and taken in ether and extracted with dil. HCl until free from quinoline. The ether layer was washed several times with water, dried over sodium sulphate and the solvent evaporated. The light brown residue obtained was passed through a column of silica gel to yield tetramethoxy stilbene as colourless oil, identical with the permethylation product of the natural sample.

Diphenylmethylenedioxy derivative of methoxy stilbene (VIII)

3,3',4-Trihydroxy-5'-methoxy stilbene (50 mg) and diphenyldichloromethane (0.05 ml) were heated at 185° for 5 minutes. The reaction mixture was cooled, dissolved in

benzene and passed through a small column of silica gel to give a solid (30 mg), crystallised from ethanol, m.p.140-141°; $C_{27}H_{22}O_4$ ($M^{+\cdot}$ m/z 410).

Gnetin (XI)

Repeated chromatography of the benzene elute of the ethyl acetate extract yielded gnetin which was crystallised from chloroform-petroleum ether as light yellow plates (100 mg), m.p.121-122°; $C_{16}H_{14}O_3$ ($M^{+\cdot}$ m/z 254).

Spectral data

| | | |
|---|---|---|
| UV(MeOH) $\lambda_{max}$ | : | 205, 302, 330 nm. |
| IR(Nujol) $\nu_{max}$ | : | 1600, 1500, 1255, 1180, 1030, 965, 930 $cm^{-1}$. |
| $^1H$ NMR(60 MHz) [$CCl_4$] | : | $\delta$ 3.80 (3H, s, OMe), 5.93 (2H, s, -O-$CH_2$-O-), 6.80-7.10 (3H, m, ArH-2,5,6), 6.82 (2H, d, J=9 Hz, ArH-3',5'), 6.87 (2H, s, -CH=CH-), 7.40 (2H, d, J=9 Hz, ArH-2',6'). |
| Mass(rel. int.) | : | m/z 254 ($M^{+\cdot}$, 100), 239 (23.5), 181 (17.7), 153 (15.8), 152 (12.5). |

Hydrogenation of gnetin

3,4-Methylenedioxy-4'-methoxy stilbene (50 mg) in methanol (20 ml) was hydrogenated over Pd/C (10%, 50 mg) for 4 hours to give a colourless oil (40 mg); $C_{16}H_{16}O_3$($M^{+\cdot}$ m/z 256).

Spectral data

IR(Nujol) $\nu_{max}$ : 1600, 1500, 1245, 1040 $cm^{-1}$.

$^1H$ NMR(60 MHz) $[CCl_4]$ : $\delta$ 2.80 (4H, s, Ph-$CH_2$-$CH_2$-Ph), 3.73 (3H, s, OMe), 6.50-7.10 (7H, m, ArH).

Synthesis of 3,4-methylenedioxy-4'-methoxy-trans-stilbene (XI)

3,4-Methylenedioxybenzaldehyde (1.5 g), para-methoxyphenyl acetic acid (1.66 g) and piperidine (0.25 ml) were heated at 160-170° for about 20 hours. The reaction mixture was cooled, dissolved in dichloromethane and filtered. The filtrate was first extracted with dil. HCl to remove piperidine and then extracted with 5% aqueous sodium hydroxide (3x20 ml) to isolate the corresponding stilbene-α-carboxylic acid (XIV). The methylenechloride layer was washed several times with water and evaporated to yield a gum (2.3 g) which was chromatographed on silica gel to give 3,4-methylenedioxy-4'-methoxy-trans-

stilbene (250 mg), found identical with the natural sample (Co-TLC, IR, NMR).

The sodium hydroxide extract was neutralised with dil. HCl and precipitated solid was collected and crystallised from methanol to give colourless needles of 3,4-methylenedioxy-4'-methoxy-trans-stilbene-α-carboxylic acid (XIV), (500 mg), m.p.252°; $C_{17}H_{14}O_5$ ($M^{+\bullet}$ m/z 298).

Spectral data

IR(Nujol) $\nu_{max}$ : 1660, 1610, 1505, 1420, 1375, 1350, 1290, 1240, 1180, 1100, 1030, 920 $cm^{-1}$.

$^1H$ NMR(60 MHz) [$CDCl_3$] : δ 3.85 (3H, s, OMe), 5.95 (2H, s, $-O-CH_2-O-$), 6.70-7.50 (8H, m, ArH; -CH=), 10.7 (1H, br s, -COOH).

Mass(rel. int.) : m/z 298 ($M^{+\bullet}$, 100), 280 (90), 265 (50), 195 (46), 152 (70), 148 (65), 126 (45), 120 (55).

Decarboxylation of 3,4-methylenedioxy-4'-methoxy-trans-stilbene-α-carboxylic acid (XIV)

3,4-Methylenedioxy-4'-methoxy-trans-stilbene-α-carboxylic acid (100 mg) was refluxed with quinoline (10 ml)

$CuCO_3$ (100 mg) for 2 hours. The cooled reaction mixture was dissolved in ether and extracted with dil. HCl until free from quinoline. The ether layer was washed several times with water, dried over sodium sulphate and the solvent evaporated. The solid obtained was crystallised from chloroform-petroleum ether to give light yellow plates of 3,4-methylenedioxy-4'-methoxy-trans-stilbene (50 mg) identical with the material obtained from plant.

Oligomeric stilbene (XV)

Elution of the column with pure ethyl acetate afforded a brown resinous mass which generally turned oily on exposure. Attempts to crystallise it failed and was, therefore, acetylated using pyridine-acetic anhydride (1:1). The product obtained after the usual work up was subjected to column chromatography over silica gel. Elution of the column with chloroform-methanol (99:1) yielded oligomeric stilbene (XV) which was crystallised from methanol as fine colourless needles (150 mg), m.p.182-183°; $C_{42}H_{36}O_{14}$ ($M^{+\cdot}$ m/z 764).

Spectral data

UV(MeOH) $\lambda_{max}$ : 205, 280, 325, 340 nm.

IR(KBr) $\nu_{max}$ : 1760, 1605, 1510, 1365, 1200, 1120, 1025, 900 $cm^{-1}$.

$^1H$ NMR(300 MHz) [$CDCl_3$] : $\delta$ 1.596 (6H, s), 1.620 (3H, s), 2.225 (6H, s), 2.241 (3H, s), 2.278 (3H, s), 2.335 (3H, s), 3.502 (3H, s, OMe), 3.711 (3H, s, OMe), 4.341 (1H, d, J=2.8 Hz), 4.408 (1H, dd, J=2.38,2.43 Hz), 6.599-6.9433 (12H, m, ArH).

Mass(rel. int.) : m/z 764 ($M^{+\bullet}$, 10), 722 (90), 680 (96), 638 (70), 596 (35), 554 (20), 512 (15), 454 (20), 432 (20), 390 (30), 368 (35), 328 (10), 313 (8), 255 (25), 158 (28).

## Maytenus emarginata

### Extraction and Isolation

Air-dried leaves (5.0 kg) of Maytenus emarginata were powdered and extracted with benzene in a Soxhlet for one week. The benzene extract was evaporated to dryness under reduced pressure to give a dark green residual mass (200 g) which was negative to specific alkaloidal reagents. The benzene exhausted powder was again dried and extracted three times with methanol at room temperature and the extract concentrated in vaccuo to yield a dark brown gummy paste (500 g) which gave positive test with both Mayers and Dragendorff reagents indicating the presence of alkaloids in this fraction. The gum was treated with 5% HCl (6x500 ml) and extracted with ethyl acetate. The ethyl acetate layer was worked up separately to isolate non-basic compounds. The aqueous acidic portion was basified with sodium bicarbonate and the precipitated solid was exhaustively extracted with chloroform, dried over sodium sulphate and the solvent removed in vaccuo leaving behind a light green residue (2.5 g). Presence of atleast four compounds was indicated by TLC plates. The crude alkaloid obtained was dissolved in minimum quantity of chloroform, adsorbed over silica gel (8.0 g), dried and then

subjected to column chromatography on the same adsorbent (30.0 g). A total of 100 fractions (20 ml each) were collected and checked on TLC plates using Dragendorff reagent.

| Fractions | Eluent | Eluate |
|---|---|---|
| 1-12 | Benzene | Fatty impurities |
| 13-20 | Benzene-$CHCl_3$ (75:25) | Fatty impurities |
| 21-30 | Benzene-$CHCl_3$ (50:50) | - |
| 31-40 | Benzene-$CHCl_3$ (25:75) | - |
| 41-55 | $CHCl_3$ | MA-2 with slight impurities |
| 56-70 | $CHCl_3$-MeOH (99:1) | MA-2 with little amount of MA-1 |
| 71-90 | $CHCl_3$-MeOH (98:2) | MA-1 with some impurity |
| 91-100 | $CHCl_3$-MeOH (95:5) | MA-1 with atleast three more compounds positive to Dragendorff reagent. |

MA-2

Fractions 41-70 were combined and the solvent evaporated in vaccuo. The light yellow coloured oil thus obtained was subjected to extensive chromatography over a column of silica gel. Prolonged elution of the column with chloroform yielded MA-2 which was found to be homogeneous on TLC plates. Several attempts to crystallise it did not succeed and it was, therefore, isolated as a white amorphous solid (125 mg), m.p.120-122°; ($M^{+\bullet}$ m/z 833).

Spectral data

IR(Nujol) $\nu_{max}$ : 1725, 1600, 1460, 1375, 1280, 1110 $cm^{-1}$.

$^1H$ NMR(300 MHz) [$CDCl_3$] : $\delta$ 1.0-3.1 (m), 4.7-6.2 (m), 6.4-9.7 (m).

Mass(rel. int.) : m/z 833 ($M^{+\bullet}$, 10), 762 (12), 716 (14), 672 (45), 610 (25), 552 (98), 228 (100), 205 (75), 160 (48), 124 (55), 105 (99).

MA-1 (XVII)

Fractions 71-90 were combined and rechromatographed on a column of silica gel (6.0 g). In all 50 fractions

(10 ml each) were collected and monitored by TLC [silica gel, methylenechloride-isopropanol-water (96:4:0.5), visualised with Dragendorff reagent]. The fractions which were eluted by chloroform-methanol (98:2) were found to contain the same component which was homogeneous on high resolution TLC plates. These fractions were combined and the solvent removed under reduced pressure leaving behind a white sticky matter (100 mg) that failed to crystallise and was, eventually, obtained as a white amorphous powder (45 mg), m.p.152-153°; $C_{25}H_{31}N_3O_2$ ($M^{+\bullet}$ m/z 405).

Spectral data

| | | |
|---|---|---|
| UV(MeOH) $\lambda_{max}$ | : | 223, 280 nm. |
| IR(KBr) $\nu_{max}$ | : | 3300, 1645, 1585, 1550, 1499, 1420, 760, 700 $cm^{-1}$. |
| $^1H$ NMR(90 MHz) $[CDCl_3]$ | : | $\delta$ 1.38-3.8 (18H, m, $-CH_2-$; -N-H), 3.85-4.0 (1H, m, H-8), 6.80 [1H, d, J=15.5 Hz, α-H(Acyl)], 7.2-7.5 (10H, m, ArH), 7.71 [1H, d, J=15.5 Hz, β-H(Acyl)]. |
| $^1H$ NMR(300 MHz) $[CDCl_3]$ | : | $\delta$ 1.2-2.7 (11H, br m, $-CH_2$; -N-H), 3.1-3.9 (7H, br m, $-CH_2$; -N-H), 3.992 |

(1H, m, $C_8$-H), 6.05 (1H, q, probably due to the acyl hydrogen of the cis isomer), 6.60 (1H, q, probably due to the acyl hydrogen of the cis isomer), 6.84 [1H, q, J=15.3,5.76 Hz, α-H(Acyl)], 7.20-7.52 (10H, m, Ar-H), 7.72 [1H, d, J=15.2 Hz, β-H(Acyl)].

Mass(rel. int.) : m/z 405 ($M^{+\circ}$, 30), 380 (50), 379 (45), 363 (18), 311 (20), 274 (80), 260 (5), 160 (30), 145 (40), 131 (45), 105 (100), 91 (20), 77 (65).

Kaempferol (XVI)

The residual mass left after the removal of alkaloids was subjected to column chromatography over silica gel (100 g). Elution of the column with benzene-ethyl acetate (80:20) supplied a greenish yellow solid which was purified by further column chromatography and repeatedly crystallised from acetone-benzene as a flocculent yellow solid (200 mg), m.p.270-272°; $C_{15}H_{10}O_6$ ($M^{+\cdot}$ m/z 286).

Spectral data

IR(KBr) $\nu_{max}$ : 3300-3400 (br), 1640, 1600, 1550, 1360, 1200, 1175, 1110, 840 $cm^{-1}$.

$^1H$ NMR(100 MHz) [DMSO-$d_6$] : $\delta$ 6.21 (1H, d, J=2 Hz, ArH-6), 6.49 (1H, d, J=2 Hz, ArH-8), 6.90 (2H, d, J=9 Hz, ArH-3',5'), 8.10 (2H, d, J=9 Hz, ArH-2',6'), 12.50 (1H, s, OH, exchangeable on addition of $D_2O$).

Mass(rel. int.) : m/z 286 ($M^{+\bullet}$, 100), 285 (25), 258 (10), 257 (8), 229 (8), 121 (25).

Acetylation of (XVI)

Kaempferol (100 mg) was acetylated by pyridine-acetic anhydride (1:1). The reaction mixture was worked up in the usual manner and the product obtained was crystallised from methanol (80 mg), m.p.182-183°.

## Maytenus emarginata (Roots)

### Extraction and Isolation

Air-dried roots of Maytenus emarginata (3.0 kg) were cut into small pieces, defatted with petroleum ether and then extracted with methanol at room temperature for ten days. Removal of the solvent under reduced pressure gave a dark red gummy paste (500 g) which was resolved into ether and ethyl acetate soluble fractions by successive extraction of its aqueous suspension in a liquid-liquid extractor. TLC plates run with benzene-ethyl acetate (85:15) showed the ether fraction to be a mixture of atleast five components. The residue obtained from the ether extract was adsorbed on silica gel and placed on a column of the same adsorbent (100 g).

### β-Amyrone (XX)

The first product to elute from the column (benzene-petroleum ether; 90:10) was initially obtained as an oily residue which was shown to be homogeneous on TLC plates. The oil was repeatedly crystallised from benzene-petroleum ether as colourless needles (600 mg), m.p.159-161°; $C_{30}H_{48}O$ ($M^{+\bullet}$ m/z 424); identified as β-amyrone by comparison with an authentic sample (mixed m.p., IR, TLC).

Spectral data

IR(KBr) $\nu_{max}$ : 2850-2950 (br), 1700, 1500 $cm^{-1}$.

$^1H$ NMR(100 MHz) $[CDCl_3]$ : $\delta$ 0.8-1.3 (each 3H, s, 8x$CH_3$), 5.3 (1H, m, -CH=C).

Mass(rel. int.) : m/z 424 ($M^{+\cdot}$, 48), 218 (99), 206 (50), 203 (100).

β-Amyrin (XXI)

The benzene-ethyl acetate (95:5) eluates were combined and processed. The solid obtained (1.5 g) was crystallised from benzene-petroleum ether as white needles, m.p.198-200°. It was identified as β-amyrin by comparison with an authentic sample.

β-Amyrin acetate

β-Amyrin (100 mg) was heated on a water bath for 4 hours with acetic anhydride and pyridine (1 ml each) and the acetate worked up was crystallised from methanol (90 mg), m.p.240-241°.

Jones oxidation of β-amyrin

Jones reagent was obtained by careful addition of conc. $H_2SO_4$ to a cold solution of chromium trioxide (70 g) in water (500 ml). A solution of β-amyrin (200 mg) in acetone (15 ml) was cooled and treated with Jones reagent till a blue colour persisted. A few drops of methanol were added to destroy excess of the reagent and then extracted with ether. The ether extract was washed several times with aqueous $K_2CO_3$, dried over sodium sulphate, evaporated and crystallised from petroleum ether as fine colourless needles (130 mg), m.p.155-156°; found identical with β-amyrone (Co-TLC, IR).

Triterpene lactone-A (XXIII)

The ethyl acetate soluble fraction (100 g) was subjected to column chromatography over silica gel (200 g). The column was first eluted with petroleum ether and then with petroleum ether-benzene (50:50) which yielded only oils and fats. Elution of the column with benzene gave β-amyrone and with benzene-chloroform (75:25) β-amyrin. Elution with chloroform-methanol (99:1) afforded a brown solid (200 mg) which was shown to be pure except for traces of some colouring matter sticking tenaciously to it. This was passed through a

small column of silica gel and then repeatedly crystallised from chloroform-petroleum ether to give a colourless crystalline solid (80 mg), m.p.327-330°; $C_{30}H_{46}O_3$ ($M^{+\cdot}$ m/z 454.3444).

Spectral data

| | | |
|---|---|---|
| UV(MeOH) $\lambda_{max}$ | : | 205 nm. |
| IR(KBr) $\nu_{max}$ | : | 3500, 1750, 1390, 1100 $cm^{-1}$. |
| $^1H$ NMR(300 MHz) [$CDCl_3$] | : | $\delta$ 0.79 (3H, s, $CH_3$-24), 0.87 (3H, s, $CH_3$-23), 0.93 (3H, s, $CH_3$-25), 0.95 (3H, s, $CH_3$-26), 0.99 (3H, s, $CH_3$-30), 1.06 (3H, s, $CH_3$-29), 1.20 (3H, s, $CH_3$-27), 3.23 (1H, m, H-3), 4.148 (1H, d, J=5.4 Hz, H-15), 5.30 (1H, t, J=3.60, 3.65 Hz, -CH=C). |
| Mass(rel. int.) | : | m/z 454.3444 ($M^{+\cdot}$, 5; required 454.3446), 285 (3), 246.16146 (100; required 246.1602), 208 (25), 202 (15), 190 (45), 119 (35). |

## Excoecharia agallocha

### Extraction and Isolation

Air dried heart wood of Excoecharia agallocha was exhaustively extracted with petroleum ether in a Soxhlet and the solvent removed in vaccuo. TLC examination of the residue (40 g) revealed the presence of two major components, both of which were revealed by spraying the plate with alcoholic solution of phosphomolybdic acid. The alkaloidal nature of the less polar of these was evident from the positive orange colour developed on spraying the plate with Dragendorff's reagent. The extract was, therefore, chromatographed on a column of silica gel (100 g). Elution with petroleum ether and benzene gave mixture of oily products which were not examined further. Prolonged elution of the column with chloroform supplied the alkaloidal and with chloroform-methanol (95:5) the non-alkaloidal components.

### Piperidine alkaloid (XXV)

The oil obtained from the chloroform eluate was highly contaminated with impurities having close $R_f$ values. It was purified through repeated chromatography over silica

gel to yield a light yellow oil (400 mg) which was found to be homogeneous on examination by TLC, $C_{21}H_{29}NO_3$ ($M^{+\circ}$ m/z 343).

Spectral data

UV(MeOH) $\lambda_{max}$ : 235, 285, 350 nm.

IR(KBr) $\nu_{max}$ : 1650, 1600, 1498, 1425, 1290, 1280, 1215, 1100, 1015, 780 $cm^{-1}$.

$^1H$ NMR(60 MHz) [$CDCl_3$] : $\delta$ 1.40-1.65 [9H, br s, $=C-CH_3$; $-(CH_2)_3-$], 1.68 (3H, s, $=C-CH_3$), 3.25 (2H, d, $Ar-C\underline{H}_2-CH=$), 3.40-3.60 [4H, br s, $-N-(CH_2-)_2$], 3.62 (3H, s, OMe), 3.72 (3H, s, OMe), 5.04 (1H, m, $-CH_2-C\underline{H}=$), 6.55 (1H, d, J=9 Hz, ArH-5), 6.70 (1H, d, J=16 Hz, $Ar-CH=C\underline{H}-CO-$), 7.20 (1H, d, J=9 Hz, ArH-6), 7.62 (1H, d, J=16 Hz, $Ar-C\underline{H}=CH-CO-$).

$^1H$ NMR(90 MHz) [$CDCl_3$+benzene-$d_6$] : $\delta$ 1.30-1.50 [6H, br s, $-(CH_2)_3-$], 1.55 and 1.66 (3H each s, 2x$=C-CH_3$), 3.15-3.50 [6H, m, $-N-(CH_2)_2$; $Ar-CH_2-CH=$], 3.58 (6H, s, 2x$OCH_3$), 5.04 (1H, m, $-CH_2-C\underline{H}=$), 6.38 (1H, d, J=9 Hz, ArH-5),

6.68 (1H, d, J=16 Hz, Ar-CH=CH-CO-), 7.16 (1H, d, J=9 Hz, ArH-6), 7.66 (1H, d, J=16 Hz, Ar-CH=CH-CO-).

Synthesis of piperidine alkaloid (XXV)

(i) Preparation of (XXVIII)

To osthol (XXVII) (300 mg) in aqueous sodium hydroxide (60%, 5 ml), dimethylsulphate (5 ml) was added dropwise with vigorous stirring. The mixture was kept on a water bath for 5 hours with occasional stirring, cooled and extracted with ether to remove unreacted osthol and dimethylsulphate. The aqueous basic layer was then acidified with dilute HCl and extracted with ether. TLC examination of the ether layer indicated it to be a mixture which was purified by column chromatography over silica gel (15.0 g) to give (XXVIII) as colourless oil (200 mg).

(ii) Conversion of (XXVIII) to (XXIX)

2,4-Dimethoxy-3-γ,γ-dimethylallyl-trans-cinnamic acid (XXVIII) was taken in dry benzene and refluxed with thionyl chloride (2 ml) in presence of traces of pyridine for 1 hour.

Evaporation of the solvent under reduced pressure supplied (XXIX) which was used for the next step.

(iii) Reaction of (XXIX) with piperidine

The acid chloride (XXIX) was dissolved in dry benzene (20 ml) and piperidine (1 ml) was added to it. The reaction mixture was refluxed on a water bath for about 1 hour, cooled and washed successively with dilute HCl until free from piperidine. The organic layer was washed several times with water, dried over sodium sulphate and the solvent evaporated to yield a gum (80 mg) which was shown to be slightly impure on TLC plates. The gum was subjected to column chromatography and elution with chloroform yielded a light yellow oil (50 mg) found homogeneous on TLC examination. The synthetic compound was found identical with the natural sample (XXV) (Co-TLC, IR, NMR).

2',4',6',4-Tetramethoxychalcone (XXIV)

The chloroform-methanol (95:5) eluates were combined, concentrated and the solid obtained was crystallised from methanol to give yellow shining plates (500 mg). It was identified as 2',4',6',4-tetramethoxychalcone by comparison with an authentic sample, m.p.140°; $C_{19}H_{20}O_5$ ($M^{+\cdot}$ m/z 328).

Spectral data

IR(Nujol) $\nu_{max}$ : 1670, 1610, 1590 $cm^{-1}$.

$^1H$ NMR(100 MHz) $[CDCl_3]$ : $\delta$ 3.66 (6H, s, 2xOMe), 3.74 and 3.72 (3H each s, 2xOMe), 6.02 (2H, s, ArH-3',5'), 6.65 (1H, d, J=16 Hz, Ar-CH=CH-CO-), 6.72 (2H, dd, J=9,2 Hz, ArH-3,5), 7.15 (1H, d, J=16 Hz, ArH-CH=CH-CO-), 7.34 (2H, dd, J=9,2 Hz, ArH-2,6).

Synthesis of (XXIV)

A solution of 2,4,6-trimethoxyacetophenone (2.10 g) and 4-methoxybenzaldehyde (1.36 g) in ethanol (95%, 20 ml) was treated with aqueous sodium hydroxide (2.0 g in 10 ml). The reaction mixture was kept at room temperature for 48 hours. It was then diluted with cold water, acidified with concentrated HCl and extracted with ether. The ether extract was washed several times with water, dried over anhydrous sodium sulphate and evaporated to yield a solid which was purified by repeatedly crystallising it with methanol, light yellow prisms (2.0 g), it was found identical with the natural sample (Co-TLC, IR, NMR).

## Cissus pallida

### Extraction and Isolation

Air dried stem wood (10 kg) of the plant was cut into small pieces and extracted twice by refluxing with petroleum ether. TLC examination of the concentrated extract (100 g) revealed it to be a mixture of several components. It was, therefore, chromatographed over a column of silica gel. Elution of the column with petroleum ether and benzene-petroleum ether afforded only oily material which was rejected. The benzene and benzene-ethyl acetate (95:5) eluates yielded two compounds CP-2 and CP-3.

The defatted plant material was extracted thrice with ethanol by percolation at room temperature. Removal of the solvent under reduced pressure left a dark brown gummy paste (250 g) which was dissolved in methanol and refrigerated. It yielded no solid and the concentrated extract was, therefore, taken up in water and exhaustively extracted with ethyl acetate in a liquid-liquid extractor. The solvent was removed in vaccuo leaving behind a brown residue (150 g). Separation of the components was brought about by column chromatography over silica gel (250 g). Three compounds were isolated and labelled as CP-1, CP-4 and CP-5.

CP-2 (Dimethyl terephthalate, XXX)

This was eluted when the column was run with benzene and was crystallised from petroleum ether-chloroform as colourless needles, m.p.125-126°; $C_{10}H_{10}O_4$ ($M^{+\cdot}$ m/z 194).

Spectral data

| | | |
|---|---|---|
| UV(MeOH) $\lambda_{max}$ | : | 250, 285 nm. |
| IR(Nujol) $\nu_{max}$ | : | 1710 $cm^{-1}$. |
| $^1H$ NMR(60 MHz) $[CCl_4]$ | : | $\delta$ 3.92 (6H, s, 2xOMe), 8.05 (4H, s, ArH). |
| Mass | : | m/z 194 ($M^{+\cdot}$), 180, 175, 166. |

Hydrolysis of dimethyl terephthalate

The methyl ester (100 mg) was dissolved in alcoholic potassium hydroxide (5 ml) and kept at room temperature for 6 hours, and the reaction mixture worked up to give a solid which was crystallised from chloroform-petroleum ether, m.p.300°; found identical when compared with an authentic sample.

CP-3 (β-Sitosterol, XXXI)

This was crystallised from methanol (1.2 g), m.p.135-137°; identified as β-sitosterol by comparison with an authentic sample (Co-TLC, mixed m.p., IR).

β-Sitosterol acetate

It was prepared by heating β-sitosterol (200 mg) with acetic anhydride and pyridine (2 ml each) on a water bath for 2 hours, and worked up to give needles; m.p.127-128°.

CP-1 (Pallidol, XXXIV)

The ethyl acetate soluble fraction was adsorbed on silica gel and subjected to column chromatography. The benzene-ethyl acetate (60:40) eluates were combined as they contained the same component. The solvent was removed under reduced pressure leaving behind a brown solid which was found homogeneous on TLC plates except for traces of some colouring matter sticking tenaciously to it. Repeated column chromatography yielded a white powder. Several attempts to crystallise it failed and it was eventually isolated as a white amorphous powder (1.8 g), m.p. >300° (with decomposition), $C_{28}H_{22}O_6$ ($M^{+\cdot}$ m/z 454.14022; required 454.1415), $[\alpha]_D$ 0° (MeOH).

Spectral data

UV(MeOH) $\lambda_{max}$ : 210, 284 nm.

IR(KBr) $\nu_{max}$ : 3350 (br), 1605, 1510, 1240, 1125, 835 $cm^{-1}$.

$^1H$ NMR(100 MHz) [$CDCl_3$+DMSO-$d_6$] : $\delta$ 3.74 (2H, br s, H-6,12), 4.43 (2H, br s, H-5,11), 6.13 (2H, d, J=2 Hz, H-1,7), 6.46 (2H, d, J=2 Hz, H-3,9), 6.63 (4H, d, J=9 Hz, H-3',5',3'',5''), 6.90 (4H, d, J=9 Hz, H-2',6',2'',6''), 8.39 (2H, s, exchangeable on addition of $D_2O$, 2xOH), 8.65 (4H, s, exchangeable on addition of $D_2O$, 4xOH).

Mass(rel. int.) : m/z 454.14022 ($M^{+\cdot}$, 18; required 454.1415), 359.09133 (18; required 359.0918), 227 (2), 171 (1), 119 (2), 94 (5).

Methylation of pallidol

A mixture of pallidol (100 mg), anhydrous potassium carbonate (3.0 g), methyl iodide (5.0 ml) was refluxed in dry acetone (100 ml) for 5 hours. The reaction mixture was filtered and the residue washed several times with hot acetone. The solvent

was evaporated to dryness under reduced pressure which left a light yellow coloured oil. The methylated product was repeatedly crystallised from methanol as colourless plates (70 mg), m.p.150-151°; $C_{34}H_{34}O_6$ ($M^{+\bullet}$ m/z 538).

Spectral data

IR(Nujol) $\nu_{max}$ : 1590, 1505, 1460, 1165, 1140, 1030, 835 $cm^{-1}$.

$^1H$ NMR(60 MHz) [$CDCl_3$] : $\delta$ 3.55 (6H, s, 2xOMe), 3.71 (6H, s, 2xOMe), 3.80 (6H, s, 2xOMe), 3.93 (2H, br s, H-6,12), 4.55 (2H, br s, H-5,11), 6.23 (2H, d, J=2 Hz, H-1,7), 6.64 (2H, d, J=2 Hz, H-3,9), 6.75 (4H, d, J=9 Hz, H-3',5',3'',5''), 7.05 (4H, d, J=9 Hz, H-2',6',2'',6'').

Mass(rel. int.) : m/z 538 ($M^{+\bullet}$, 100), 430 (95), 417 (55), 399 (65), 323 (24), 269 (29), 255 (18), 240 (15), 215 (18), 121 (70).

Pallidol hexaacetate

A mixture of pallidol (100 mg), pyridine (1 ml) and acetic anhydride (1 ml) was kept overnight at room temperature,

and the reaction mixture worked up in the usual manner. The white sticky material obtained was repeatedly crystallised from chloroform-petroleum ether as colourless needles (75 mg), m.p.126-127°; $C_{40}H_{34}O_{12}$ ($M^{+\cdot}$ m/z 706), $[\alpha]_D$ 0° ($CHCl_3$).

Spectral data

IR(KBr) $\nu_{max}$ : 1760, 1620, 1600, 1510, 1480, 1440, 1380, 1310, 1220, 1130, 1060, 1030, 930, 870 $cm^{-1}$.

$^1H$ NMR(90 MHz) [$CDCl_3$] : $\delta$ 1.70 (6H, s, 2xOAc, C-4,10), 2.29 (12H, s, 4xOAc, C-2,8,4',4''), 4.15 (2H, t, J=2.5 Hz, H-6,12), 4.45 (2H, t, J=2.5 Hz, H-5,11), 6.75 (2H, d, J=2 Hz, H-1,7), 6.85 (2H, d, J=2 Hz, H-3,9), 6.95 (4H, d, J=8 Hz, H-3',5',3'',5''), 7.15 (4H, d, J=8 Hz, H-2',6',2'',6'').

$^1H$ NMR(300 MHz) [$CDCl_3$] : $\delta$ 1.688 (6H, s, 2xOAc, C-4,10), 2.276 (6H, s, 2xOAc, C-2,8), 2.286 (6H, s, 2xOAc, C-4',4''), 4.168 (2H, dd, J=3.7, 2.6 Hz, H-6,12 i.e. $H_B, H_{B'}$), 4.448 (2H, dd, J=3.7,2.6 Hz, H-5,11 i.e. $H_A, H_{A'}$), 6.766 (2H, d, J=2.07 Hz, H-1,7), 6.884 (2H, d, J=2.05 Hz, H-3,9), 7.049 (4H, d,

J=8.46 Hz, H-3',5',3'',5''), 7.154 (4H, d, J=8.55 Hz, H-2',6',2'',6'').

Mass(rel. int.) : m/z 706 ($M^{+\cdot}$, 25), 664 (69), 622 (90), 580 (100), 538 (93), 496 (50), 454 (30), 359 (85), 347 (45), 227 (40), 107 (70).

CP-4 (β-Sitosterol glucoside, XXXII)

This compound was obtained when the column was run with benzene-ethyl acetate (25:75) as a light green solid which was repeatedly crystallised from chloroform-methanol (1.0 g), m.p.273-275°.

Spectral data

IR(Nujol) $\nu_{max}$ : 3450 $cm^{-1}$.

Mass : 414 ($M^{+\cdot}$ -179), 396, 303.

Acetylation of (XXXII)

β-Sitosterol glucoside (250 mg) was dissolved in pyridine-acetic nhydride (1:1) and heated on a water bath for 6 hours. Work up of the reaction mixture in the usual way afforded the acetylated product which was repeatedly crystallised from methanol, m.p.150-152°.

Spectral data

$^1$H NMR(300 MHz) [$CDCl_3$] : $\delta$ 0.68 (3H, s, $CH_3$), 0.80 (3H, s, $CH_3$), 0.82 (3H, s, $CH_3$), 0.98 (3H, s, $CH_3$), 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 2.04 (3H, s, OAc), 2.07 (3H, s, OAc), 3.48 (1H, m), 4.59 (1H, d, J=7.7 Hz), 5.36 (1H, br s).

Mass(rel. int.) : 396 (65), 382 (20), 331 (25), 296 (18), 255 (15), 169 (100), 108 (60).

Hydrolysis of β-sitosterol glucoside

150 mg of (XXXII) was refluxed in a mixture of HCl (2 ml) and acetic acid (1 ml) for 2 hours. The solid obtained on completion of hydrolysis was filtered, washed several times with water, dissolved in chloroform, dried over sodium sulphate and crystallised from methanol, m.p.137-139°; identified as β-sitosterol (Co-TLC, mixed m.p., IR).

CP-5 (Arjunolic acid, XXXIII)

Elution of the column with pure ethyl acetate afforded a light brown solid which was repeatedly crystallised from acetone-ethyl acetate as colourless plates (600 mg), m.p.332-334°; $C_{30}H_{48}O_5$ ($M^{+\cdot}$ m/z 488).

Spectral data

IR(KBr) $\nu_{max}$ : 3450, 2950, 1702, 1460, 1390, 1280, 1045 $cm^{-1}$.

Mass(rel. int.) : m/z 488 ($M^{+\cdot}$, 18), 452 (20), 444 (15), 249 (25), 248 (100), 204 (25), 203 (96).

Acetylation of (XXXIII)

CP-5 (100 mg) was acetylated by pyridine-acetic anhydride (1:1) and crystallised from methanol (70 mg), m.p.175-178°; $C_{36}H_{52}O_8$ ($M^{+\cdot}$ m/z 612).

Spectral data

$^1H$ NMR(400 MHz) $[CDCl_3]$ : $\delta$ 0.90 (3H, s, $CH_3$-30), 0.92 (3H, s, $CH_3$-29), 1.03 (3H, s, $CH_3$-26), 1.08 (3H,s, $CH_3$-25), 1.27 (3H, s, $CH_3$-27), 1.45 (3H,s, $CH_3$-24), 1.99 (3H, s, OAc), 2.05 (3H, s, OAc), 2.08 (3H, s, OAc), 3.72 (1H, d, J=12 Hz, $CH_2$-OAc), 3.93 (1H, d, J=12 Hz, $CH_2$-OAc), 5.00 (1H, d, J=10 Hz, H-3), 5.22 (1H, m, H-2), 5.31 (1H, t, -CH=C).

Mass(rel. int.) : m/z 612 ($M^{+\cdot}$, 5), 584 (20), 552 (10), 450 (40), 432 (40), 248 (100), 203 (98).

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# A PIPERIDINE ALKALOID FROM *EXCOECHARIA AGALLOCHA*

SATYA PRAKASH, MUSHTAQ A. KHAN, HAFIZULLAH KHAN and ASIF ZAMAN

Department of Research in Unani Medicine and Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India

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**Key Word Index** *Excoecharia agallocha*; Euphorbiaceae; 2,4-dimethoxy-3-$\psi$,$\psi$-dimethylallyl-*trans*-cinnamoylpiperidide; 2',4',6',4-tetramethoxychalcone.

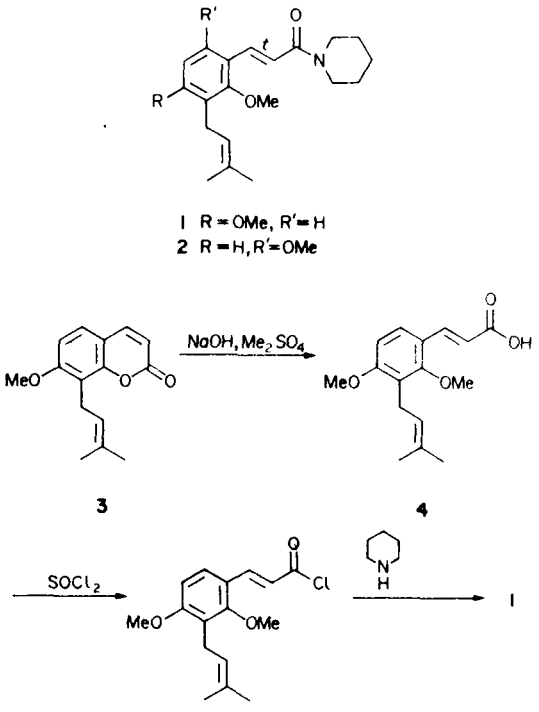
**Abstract**—Isolation of a new piperidine alkaloid and 2',4',6',4-tetramethoxychalcone from *Excoecharia agallocha* is reported. The structure 2,4-dimethoxy-3-$\psi$,$\psi$-dimethylallyl-*trans*-cinnamoylpiperidide assigned to the alkaloid was confirmed through synthesis.

*Excoecharia agallocha* L. (Euphorbiaceae) is native to Goa [1] and is used there as fish poison when the more potent *Holigarna arnottiana* (Anacardiaceae) is not available. Work-up of its stemwood afforded a crystalline compound and an oil. The $^1$H NMR spectrum of the crystalline compound agrees with that of 2',4',6',4-tetramethoxychalcone and this identification was confirmed through comparison with the permethylation product of the corresponding tetrahydroxychalcone.

The oil could not be induced to crystallize but was homogeneous on TLC. It gives a positive test with the Dragendorff reagent, shows an amide carbonyl band in the IR spectrum at 1650 cm$^{-1}$ and UV maxima at the same values as in cinnamates [2]. These structural features are confirmed by the $^1$H NMR spectrum which has singlets of two methoxyls at $\delta$ 3.62 and 3.72, doublets of two aromatic protons at 6.55 and 7.20 ($J$ = 9 Hz) and *trans* [3] olefinic protons at 6.70 and 7.62 ($J$ = 16 Hz), the one at higher field overlapping with the doublet of the aromatic proton. The remaining position in the benzene ring is occupied by a 3,3-dimethylallyl side chain of which only the methine triplet at $\delta$ 5.04 is clearly visible. The signals of the methylenes and *gem*-dimethyl groups at higher field are distorted by broad 4H and 6H signals at $\delta$ 3.40–3.60 and 1.40–1.65. These chemical shifts are the same as reported for the ring methylenes in cinnamoylpiperidides [4].

With regard to location of substituents only those structures are possible for the alkaloid which allow for two protons in *ortho* relationship and the choice is narrowed to **1** and **2** by the fact that the doublet of one aromatic proton appears at a value which is possible only if it is *ortho* and *para* to two methoxyls [5]. The *ortho* relationship of one methoxyl to a proton is easily established through a benzene induced shift [6]. The spectrum in the presence of benzene is helpful further in resolving neatly the signals of the $\psi$,$\psi$-dimethylallyl side chain and the piperidine ring methylenes.

Biogenetic considerations strongly favoured **1** and this structure was confirmed through synthesis. Methylative ring opening of osthol (**3**) according to the procedure of Divakar and Rao [7], gave 2,4-dimethoxy-3-$\psi$,$\psi$-dimethylallyl-*trans*-cinnamic acid (**4**) which was converted to the corresponding chloride, **5**, the reaction of which with piperidine yielded a compound identical in all respects with the natural sample (Scheme 1). Cinnamoylpiperidides have been encountered so far only in the genus *Piper* (Piperaceae). Isolation of **1** from a member of the Euphorbiaceae is, therefore, of some interest.




1 R = OMe, R' = H
2 R = H, R' = OMe

3 4 5

Scheme 1.

## EXPERIMENTAL

*Extraction and isolation.* Air-dried stemwood (4 kg) of *E. agallocha* (identified by Dr. Peerzada S. H. Khan, Scientist, National Botanical Research Institute, Lucknow) was extracted with petrol in a Soxhlet and the residue (40 g) obtained on evaporation of the extract was chromatographed over Si gel. Elution with petrol and $C_6H_6$ removed the fatty material and the column was then run with $CHCl_3$ and $CHCl_3$–MeOH (95:5).

2,4-*Dimethoxy*-3-$\psi$,$\psi$-*dimethylallyl*-trans-*cinnamoylpiperidide* (**1**). The impure oil obtained from the $CHCl_3$ eluate was further purified through repeated chromatography to give TLC pure material (400 mg), $[M]^+$ *m/z* 343, $C_{21}H_{29}NO_3$; IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1650, 1600, 1498, 1425, 1290, 1280, 1215, 1100, 1015 and 780; UV $\lambda_{max}^{MeOH}$ nm: 235, 285, 350; $^1$H NMR (60 MHz, $CDCl_3$): $\delta$ 7.62 (1H, *d*, *J* = 16 Hz, Ar C<u>H</u>=CH CO ), 7.20 (1H, *d*, *J* = 9 Hz, ArH-6), 6.70 (1H, *d*, *J* = 16 Hz, Ar–CH=C<u>H</u>–CO–), 6.55 (1H, *d*, *J* = 9 Hz, ArH-5), 5.04 (1H, *m*, –$CH_2$–C<u>H</u>=), 3.62 and 3.72 (3H each *s*, 2X-OMe), 3.40–3.60 [4H, *br s*, N–(C<u>H</u>$_2$–)$_2$], 3.25 (2H, *d*, Ar–C<u>H</u>$_2$–CH=), 1.68 (3H, *s*, =C–Me), 1.40–1.65 [9H, *br s*, =C–Me and –$(CH_2)_3$–]. $^1$H NMR (90 MHz, $CDCl_3$ + 8 drops of $C_6D_6$): $\delta$ 7.66 (1H, *d*, *J* = 16 Hz, Ar–C<u>H</u>=CH–CO–), 7.16 (1H, *d*, *J* = 9 Hz, ArH-6), 6.68 (1H, *d*, *J* = 16 Hz, Ar CH=C<u>H</u> CO ), 6.38 (1H, *d*, *J* = 9 Hz, ArH-5), 5.04 (1H, *m*, –$CH_2$–C<u>H</u>=), 3.58 (6H, *s*, 2X –OMe), 3.15–3.50 [6H, *m*, –N–$(CH_2)_2$– and Ar $CH_2$ $CH_2$=], 1.55 and 1.66 (3H each *s*, 2X =C Me), 1.30–1.50 [6H, *br s*, $(CH_2)_3$–].

2,4-*Dimethoxy*-3-$\psi$,$\psi$-*dimethylallyl*-trans-*cinnamic acid* (**4**). To osthol (**3**) (300 mg) in aq. NaOH (60%, 5 ml), $Me_2SO_4$ (5 ml) was added drop-wise with vigorous stirring. The mixture was kept on a water bath for 5 hr with occassional stirring, cooled and extracted with $Et_2O$ to remove unreacted coumarin and excess $Me_2SO_4$. The aq. layer was then acidified with dilute HCl and extracted with $Et_2O$. Evaporation of $Et_2O$ yielded an oil which was purified by CC on Si gel to give **4** as colourless oil (200 mg).

2,4-*Dimethoxy*-3-$\psi$,$\psi$-*dimethylallyl*-trans-*cinnamoyl chloride* (**5**). The *trans*-cinnamic acid, **4** (100 mg), in dry $C_6H_6$ (10 ml) was refluxed with $SOCl_2$ (2 ml) in presence of traces of pyridine for 1 hr. Evaporation of the solvent under red. pres. gave a viscous mass which was used for the next step.

2,4-*Dimethoxy*-3-$\psi$, $\psi$-*dimethylallyl*-trans-*cinnamoylpiperidide* (**1**). The acid chloride, **5**, in dry $C_6H_6$ (20 ml) and piperidine (1 ml) was refluxed on a water bath for 30 min. The reaction mixture was cooled and washed successively with dilute HCl until free from piperidine. The organic layer was washed several times with $H_2O$, dried and the solvent evaporated to yield a gum which was purified by CC to give a light yellow oil (50 mg) which was found to be identical with the natural sample (Co-TLC, IR).

2′,4′,6′,4-*Tetramethoxychalcone*. Crystallized from MeOH as pale yellow prisms (500 mg), mp 140°, $[M]^+$ *m/z* 328, $C_{19}H_{20}O_5$; $^1$H NMR (100 MHz, $CDCl_3$): $\delta$ 7.34 (2H, *dd*, *J* = 9, 2 Hz, ArH-2, ArH-6), 7.15 (1H, *d*, *J* = 16 Hz, Ar CH=CH CO ), 6.72 (2H, *dd*, *J* = 9, 2 Hz, ArH-3, ArH-5), 6.65 (1H, *d*, *J* = 16 Hz, Ar CH=C<u>H</u> CO ), 6.02 (2H, *s*, ArH-3′, ArH-5′), 3.74 and 3.72 (3H each *s*, 2X OMe), 3.66 (6H, *s*, 2X OMe).

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# STILBENES OF *GNETUM ULA*

SATYA PRAKASH, MUSHTAQ A. KHAN, KHALIQUZ ZAMAN KHAN and ASIF ZAMAN

Department of Research in Unani Medicine and Department of Chemistry, Aligarh Muslim University, Aligarh-202001, India

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**Key Word Index** *Gnetum ula*; Gnetaceae; stilbenes; structure; synthesis; structure revision

**Abstract**—Gnetin, a new stilbene isolated from *Gnetum ula* is assigned the structure 3,4-methylenedioxy-4'-methoxy-*trans*-stilbene, **3** on the basis of spectroscopic data and synthesis. The structure 3,3',4-trihydroxy-2-methoxy-*trans*-stilbene, **1a** earlier assigned to a trihydroxymonomethoxy stilbene is now revised to 3,4,5'-trihydroxy-3'-methoxy-*trans*-stilbene, **1b**.

## INTRODUCTION

We have earlier reported [1, 2] the isolation of two stilbenes, together with bergenin, 2-hydroxy-4-benzyloxy acetophenone and bis-2-(2,2',4,4'-tetrahydroxy)-acetophenone. These were identified as 3,3',4-trihydroxy-2-methoxy-*trans*-stilbene (**1a**) and 2,3',5',6-tetrahydroxy-*trans*-stilbene (gnetol, **2**). A third stilbene, gnetin, has now been obtained in small amounts from the combined benzene eluates of several columns and identified as 3,4-methylenedioxy-4'-methoxy-*trans*-stilbene (**3**) on the basis of spectral data discussed below and synthesis. Some fresh observations made it necessary to check the structure **1a** assigned to the methoxy stilbene and it has now been revised to **1b**.

## RESULTS AND DISCUSSION

The presence of methylenedioxy and OMe groups in gnetin was evident from 2 and 3H singlets at 5.93 and 3.80, respectively, in its $^1$H NMR spectrum. While the pattern of multiplets of the aromatic protons definitely located the OMe group it left open the possibility of fusion of the methylenedioxy group to C-2 and C-3 of the stilbene nucleus. Confirmation of **3** was obtained by synthesis through condensation [3] of piperonal with *para*-methoxyphenylacetic acid.

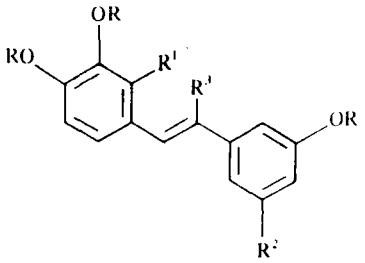
The structure of the methoxy stilbene reported earlier was based on the presence of a fragment at *m/z* 107 (100%) in the mass spectrum of the dihydro derivative and two *ortho* coupled doublets in the $^1$H NMR spectrum. The mono substitution of one benzene ring and the presence of substituents on three contiguous carbon atoms in the other therefore did not seem open to doubt. The 360 MHz spectrum of gnetol obtained later made it possible to assign resonances of all the aromatic protons in gnetol (**2**) with certainty, the assignments being then checked through benzene induced shifts. Comparison of $^1$H NMR spectra of the two compounds made structure **1a** doubtful for the methoxy stilbene. 2,3,3',4-Tetramethoxy-*trans*-stilbene (**1c**) was therefore synthesised and comparison revealed it to be different from the permethyl derivative of the natural compound. A similar comparison with synthetic 3,3',4,5'-tetramethoxy-*trans*-stilbene (**1d**) then showed that the trihydroxymonomethoxy stilbene had identical substitution. The only explanation of the parent peak at *m/z* 107 is that it arises through loss of formaldehyde. Also notable is that one of the *ortho* coupled doublets changes to a double doublet at 270 MHz. The green ferric colour suggested that the OMe group is located in the ring having a resorcinol type substitution and this was confirmed through formation of the diphenylmethylenedioxy derivative on reaction with dichloro diphenylmethane [4]. The structure of the stilbene must therefore be revised to **1b**. 3,3',4-Trimethoxy-*trans*-stilbene (**1e**) was also synthesised as a model compound and since it is not known is reported.

## EXPERIMENTAL

*Isolation of gnetin* (**3**). Defatted stem-wood of *G. ula* (5 kg) was cut into small pieces and extracted in a Soxhlet with $Me_2CO$. The residue obtained after removal of solvent under red. pres. was taken up in $H_2O$ and exhaustively extracted with EtOAc in a liquid liquid extractor. The EtOAc sol fraction (25 g) was chromatographed and elution with $C_6H_6$ gave a viscous mass which was repeatedly fractionated on silica gel to give **3** (100 mg), light yellow plates from $CHCl_3$–petrol, mp 121–122°; MS [M]$^+$ *m/z* 254 ($C_{16}H_{14}O_3$); $\nu_{max}$ (Nujol) 1600, 1500, 1255, 1180, 1030, 965, 930 cm$^{-1}$; $\lambda_{max}$ (MeOH) 205, 302 and 330 nm; $^1$H NMR ($CCl_4$, 60 MHz) 7.40 (2H, *d*, *J* = 9 Hz, Ar H-2',6'), 6.87 (2H, *s*, CH=CH), 6.82 (2H, *d*, *J* = 9 Hz, Ar H-3',5'), 6.80–7.10 (3H, *m*, Ar H-2, 5, 6), 5.93 (2H, *s*, $OCH_2O$), 3.80 (3H, *s*, OMe); MS *m/z* 254 (100), 239 (23.5), 181 (17.7), 153 (15.8), 152 (12.5).

*Dihydrognetin*. **3** (50 mg) in MeOH (20 ml) was hydrogenated over Pd/C (10%, 50 mg) for 4 hr to give a colourless oil (40 mg). $\nu_{max}$ (Nujol) 1600, 1500, 1245, 1040 cm$^{-1}$; $^1$H NMR ($CCl_4$, 60 MHz) 6.50–7.10 (7H, *m*, ArH), 5.80 (2H, *s*, $OCH_2O$), 3.73 (3H, *s*, OMe), 2.80 (4H, *s*, Ph ($CH_2$)$_2$).

*Synthesis of* **3**. Piperonal (1.5 g), *p*-methoxyphenylacetic acid (1.66 g) and piperidine (0.25 ml) were heated at 160–170° for 15 hr. The reaction mixture was cooled, dissolved in $CH_2Cl_2$ and filtered. The filtrate was first extracted with dil HCl to remove piperidine and then extracted with 5% aq NaOH (3 × 20 ml) to isolate the corresponding stilbene-$\beta$-carboxylic acid **4**. The $CH_2Cl_2$ layer was washed several times with $H_2O$ and evapd to yield a gum (2.3 g) which was chromatographed on silica gel to give **3** (250 mg), found identical with the natural sample (co-TLC, IR, $^1$H NMR).



**1a** $R = R^2 = R^3 = H$, $R^1 = OMe$

**1b** $R = R^1 = R^3 = H$, $R^2 = OMe$

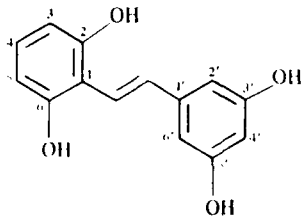
**1c** $R^2 = R^3 = H$, $R = Me$, $R^1 = OMe$

**1d** $R^1 = R^3 = H$, $R = Me$, $R^2 = OMe$

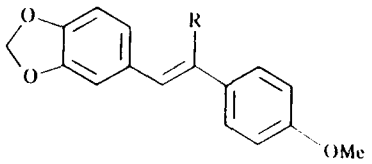
**1e** $R^1 = R^2 = R^4 = H$, $R = Me$

**1f** $R^1 = R^2 = H$, $R = Me$, $R^4 = COOH$

**1g** $R^1 = H$, $R = Me$, $R^2 = OMe$, $R^4 = COOH$



**2**



**3** R = H

**4** R = COOH

The NaOH extract was neutralised with dil HCl and ppted solid was collected and crystallised from MeOH to give colourless needles of **4** (500 mg), mp 252°; MS $[M]^+$ *m/z* 298 ($C_{17}H_{14}O_5$); $\nu_{max}$ (Nujol) 1660, 1610, 1600, 1505, 1420, 1375, 1350, 1290, 1240, 1180, 1100, 1030, 920 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 60 MHz) 10.7 (1H, *br s*, COOH), 6.70–7.50 (8H, *m*, ArH and -CH=), 5.95 (2H, *s*, O $CH_2$ O), 3.85 (3H, *s*, OMe); MS *m/z* 298 (100), 280 (90), 265 (50), 195 (46), 152 (70), 148 (65), 126 (45), 120 (55).

*Decarboxylation of* **4**. **4** (100 mg) was refluxed with quinoline (10 ml) and $CuCO_3$ [5] (100 mg) for 2 hr. The cooled reaction mixture was dissolved in $Et_2O$ and extracted with dil HCl until free from quinoline. The solid obtained after usual work up of the $Et_2O$ layer was crystallised from $CHCl_3$–petrol to give plates of **3** (50 mg), identical with the material obtained from plant.

3,4,5′-*Trihydroxy*-3′-*methoxy*-trans-*stilbene* (**1b**). Isolation according to the procedure in ref. [1]. $^1H$ NMR (DMSO-$d_6$, 270 MHz) 9.50 (3H, *br s*, exchangeable on addition of $D_2O$, 3 × -OH), 7.16 (1H, *d*, *J* = 2 Hz, ArH-5), 6.96 (1H, *dd*, *J* = 8, 2 Hz, ArH-6), 6.90 (2H, *dd*, *J* = 17 Hz, CH=CH), 6.75 (1H, *d*, *J* = 8 Hz, ArH-2), 6.40 (2H, *d*, *J* = 2 Hz, ArH-2′,6′), 6.12 (1H, *br s*, ArH-4′), 3.82 (3H, *s*, OMe); $^1H$ NMR (DMSO-$d_6$ + $C_6D_6$, 270 MHz) 9.40 (3H, *br s*, exchangeable on addition of $D_2O$, 3 × -OH), 7.20 (1H, *d*, *J* = 2 Hz, ArH-5), 6.99 (1H, *dd*, *J* = 8, 2 Hz, ArH-6), 6.98 (2H, *dd*, *J* = 17 Hz, CH=CH), 6.83 (1H, *d*, *J* = 8 Hz, ArH-2), 6.53 (2H, *d*, *J* = 2 Hz, ArH-2′,6′), 6.28 (1H, *dd*, *J* = 2 Hz, ArH-4′), 3.83 (3H, *s*, OMe).

2,3,3′,4-*Tetramethoxy*-trans-*stilbene* (**1c**). A mixture of 2,3,4-trimethoxybenzaldehyde (1.96 g), *m*-methoxyphenyl acetic acid (1.66 g) and piperidine (0.25 ml) was heated at 160–170° for 20 hr. The reaction mixture was dissolved in $CH_2Cl_2$ and worked up as before to give a gum (1.5 g). Chromatographic separation of this material on silica gel gave **1c** as a colourless oil (500 mg). MS $[M]^+$ *m/z* 300 ($C_{18}H_{20}O_4$); $^1H$ NMR ($CDCl_3$, 60 MHz) 6.2–7.4 (8H, *m*, ArH and CH=CH), 3.40, 3.50, 3.80 and 3.85 (3H each *s*, 4 × OMe).

*Synthesis of* 3,3′,4-*trimethoxy*-trans-*stilbene* (**1e**). Condensation of 3,4-dimethoxybenzaldehyde (1.66 g) and 3-methoxyphenyl acetic acid (1.66 g) as above gave **1e** (300 mg) along with the corresponding stilbene-β-carboxylic acid **1f** (700 mg) from the NaOH extract. **1e**: colourless oil; MS $[M]^+$ *m/z* 270 ($C_{17}H_{18}O_3$); $^1H$ NMR ($CDCl_3$, 60 MHz) 6.7–7.6 (9H, *m*, ArH and -CH=CH-), 3.60, 3.90 and 4.0 (3H each *s*, 3 × $-OCH_3$). **1f**: Colourless needles from MeOH, mp 200–201°; MS $[M]^+$ *m/z* 314 ($C_{18}H_{18}O_5$); $^1H$ NMR ($CDCl_3$, 60 MHz) 10.7 (1H, *br s*, exchangeable on addition of $D_2O$, COOH), 6.5–7.5 (9H, *m*, ArH and CH=), 3.70, 3.90 and 3.95 (3H, each *s*, 3 × OMe).

*Synthesis of* 3,3′,4,5′-*tetramethoxy*-trans-*stilbene* (**1d**). Condensation of 3,4-dimethoxybenzaldehyde with 3,5-dimethoxyphenyl acetic acid [6] (1 mmol each) in presence of piperidine (0.25 ml) at 160–170° for 20 hr gave **1d** (300 mg) in the neutral and **1g** (600 mg) in the alkaline fraction. **1d**: colourless oil; MS $[M]^+$ *m/z* 300 ($C_{18}H_{20}O_4$); $^1H$ NMR ($CDCl_3$, 60 MHz) 6.0–7.0 (8H, *m*, ArH and CH=CH), 3.85 (3H, *s*, OMe), 3.80 (9H,

s, 3 × OMe). Found identical with the permethyl ether of natural **1b**. **1g**: colourless needles from MeOH, mp 180–182°; MS $[M]^+$ *m/z* 344 ($C_{19}H_{20}O_6$); $^1H$ NMR ($CDCl_3$, 60 MHz) 10.8 (1H, *br s*, exchangeable on addition of $D_2O$, –COOH), 6.0–7.5 (7H, *m*, ArH and –CH=), 4.10 (6H, *s*, 2 × OMe), 4.15 (6H, *s*, 2 × –OMe).

*Diphenylmethylenedioxy derivative of* **1b**. **1b** (50 mg) and diphenyldichloromethane (0.05 ml) were heated at 185° for 5 min. The reaction mixture was cooled, dissolved in $C_6H_6$ and passed through a small column of silica gel to give a solid (30 mg), crystallised from EtOH, mp 140–141°, MS $[M]^+$ *m/z* 410 ($C_{27}H_{22}O_4$).

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# THREE 3-BENZYL-4-CHROMANONES FROM *MUSCARI COMOSUM*

MATTEO ADINOLFI, GASPARE BARONE, ROSA LANZETTA, GUGLIELMO LAONIGRO, LORENZO MANGONI and MICHELANGELO PARRILLI*

Dipartimento di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, 80134 Napoli, Italy

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**Key Word Index**—*Muscari comosum*; Liliaceae; bulbs; 3-benzyl-4-chromanones; homoisoflavanones; 5,8-dihydroxy-3-(4′-hydroxybenzyl)-6,7-dimethoxy-4-chromanone; 5,7-dihydroxy-3-(3′-hydroxy-4′-methoxybenzyl)-4-chromanone; 5,7-dihydroxy-3-(4′-hydroxybenzyl)-4-chromanone.

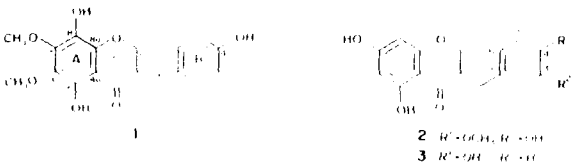
**Abstract**—Three novel 3-benzyl-4-chromanones have been isolated from the bulbs of *Muscari comosum*.

## INTRODUCTION

We recently [1] described the structural elucidation of three components of the homoisoflavanone fraction extracted from the bulbs of *Muscari comosum*. In the present paper we report the spectral data which now allow us to assign structures **1**, **2** and **3** to a further three homoisoflavanones from the same source, named muscomin, 3′-hydroxy-3,9-dihydroeucomin and 4′-demethyl-3,9-dihydroeucomin, respectively. It is noteworthy that **1** and **2**, as compared to known 3-benzyl-4-chromanones [2], possess new oxygenation patterns. Compound **1** bears oxygen functions at both positions 6 and 8 of ring A in addition to the normally oxygenated functions 5 and 7, and compound **2** bears a hydroxyl group at the 3′ position like scillascillins, although it does not possess the 3-spirocyclobutene ring which is characteristic of these compounds.

## RESULTS AND DISCUSSION

Compound **1** possesses the molecular formula $C_{18}H_{18}O_7$ (high-resolution mass spectrum). In the $^1H$ NMR spectrum the signals of the protons of rings B and C were clearly seen (Table 1). The remaining resonances were those of three hydroxyl and two methoxyl groups. The appearance of the hydroxytropylium fragment (*m/z* 107) in the mass spectrum indicated that one hydroxyl group was at the 4′ position. It was assigned the $\delta$9.31 $^1H$ NMR signal because an NOE was measured between this and the 3′,5′ signals. The UV absorption at



1



2 R′=OCH, R″=OH
3 R′=OH, R″=H

*To whom correspondence should be addressed.